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EP 0 132 919 B1

## Description

This invention relates to novel peptides.

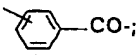
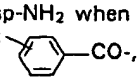
As regards peptides analogues to the peptide of the invention, there is known for example a peptide represented by formula

Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$

(hereinafter this peptide is referred to as «CCK-8»). CCK-8 is known to have a wide variety of physiological actions such as the contraction of gallbladder, stimulation of pancreatic enzyme secretion, stimulation of pancreatic internal secretion, potentiation of intestinal movement, depression of gastric secretion, pancreas proliferation, depression of central nervous system, sedation, enhancement of sleeping time, anti-psychopathia action, anti convulsive action, analgesia, and anorexia.

It is an object of the present invention to provide novel peptides of the following formula (1) and pharmacologically acceptable salts thereof, and a process by which they can
be obtained in a high yield and high purity,

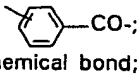
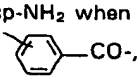
$R_1$-A-B-C-D-Trp-Met-Y (1)

wherein; $R_1$ denotes pGlu, X-$R_2$-CO-, X being carboxyl or amino and $R_2$ being lower alkylene of 1-6 carbon atoms, or  ; A denotes Asp, Ala, or merely a chemical bond; B denotes Tyr($SO_3H$) or Phe($NHSO_3H$); C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes $NH_2$, Asp-$NH_2$, or Asp-Phe-$NH_2$; with the proviso that Y is $NH_2$ or Asp-$NH_2$ when $R_1$ is pGlu, HOOC-$R_2$-CO-, or  , B is Tyr($SO_3H$), C is Met, and D is Gly.

It is another object of the present invention to provide a pharmaceutical composition useful for accelerating the pancreatic function, which comprises an effective amount of formula (1) or pharmacologically acceptable salts thereof.

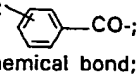
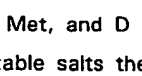
According to one aspect of the present invention, there is provided novel peptides represented by the following formula

$R_1$-A-B-C-D-Trp-Met-Y (1)

wherein; $R_1$ denotes pGlu, X-$R_2$-CO-, X being carboxyl or amino and $R_2$ being lower alkylene of 1-6 carbon atoms, or  ; A denotes Asp, Ala, or merely a chemical bond; B denotes Tyr($SO_3H$) or Phe($NHSO_3H$); C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes $NH_2$, Asp-$NH_2$, or Asp-Phe-$NH_2$; with the proviso that Y is $NH_2$ or Asp-$NH_2$ when $R_1$ is pGlu, HOOC-$R_2$-CO-, or  , B is Tyr($SO_3H$), C is Met, and D is Gly, and pharmacologically acceptable salts thereof.

According to another aspect of the present invention, there is provided a process for the preparation of novel peptides represented by the following formula

$R_1$-A-Tyr($SO_3H$)-C-D-Trp-Met-Y (6)

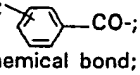
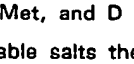
wherein; $R_1$ denotes pGlu, X-$R_2$-CO-, X being carboxyl or amino and $R_2$ being lower alkylene of 1-6 carbon atoms, or  ; A denotes Asp, Ala, or merely a chemical bond; C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes $NH_2$, Asp-$NH_2$, or Asp-Phe-$NH_2$; with the proviso that Y is $NH_2$ or Asp-$NH_2$ when $R_1$ is pGlu, HOOC-$R_2$-CO-, or  , C is Met, and D is Gly, and pharmacologically acceptable salts thereof, characterized in that the Tyr-residue in the peptide of the following formula

$R_1$-A-Tyr-C-D-Trp-Met-Y (2)

wherein; $R_1$, A, C, D, and Y are the same as defined above, or the peptide in which active groups such as amino groups are protected by suitable protecting groups is subjected to the sulfate-ester reaction to convert the Tyr-residue to Tyr($SO_3H$) residue.

According to further an aspect of the present invention, there is provided a process for the preparation of novel peptides represented by the following formula

$R_1$-A-Tyr($SO_3H$)-C-D-Trp-Met-Y (6)

wherein; $R_1$ denotes pGlu, X-$R_2$-CO-, X being carboxyl or amino and $R_2$ being lower alkylene of 1-6 carbon atoms, or  ; A denotes Asp, Ala, or merely a chemical bond; C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes $NH_2$, Asp-$NH_2$, or Asp-Phe-$NH_2$; with the proviso that Y is $NH_2$ or Asp-$NH_2$ when $R_1$ is pGlu, HOOC-$R_2$-CO-, or  , C is Met, and D is Gly, and pharmacologically acceptable salts thereof, characterized in that a sulfo group of a protected peptide amide sulfate ester obtained by the sulfate-ester reaction of the Tyr residue in the protected peptide of the following formula

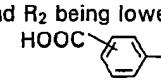
$R_1$-A-Tyr-C-D-Trp-Met-Y (2)

wherein; $R_1$, A, C, D, and Y are the same as defined above, is converted to a salt of divalent metals such as Ca, Zn, and the like to stabilize said protected peptide amide sulfate ester, and thereafter said protected peptide amide sulfate ester is deprotected.

According to still further an aspect of the present invention, there is provided a process for the pre-

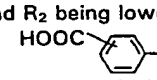
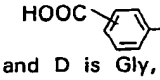
paration of novel peptides represented by the following formula

$R_1$-A-Phe($NHSO_3H$)-C-D-Trp-Met-Y (6)

wherein; $R_1$ denotes pGlu, X-$R_2$-CO-, X being carboxyl or amino and $R_2$ being lower alkylene of 1-6 carbon atoms, or  ; A denotes Asp, Ala, or merely a chemical bond; C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp, and pharmacologically acceptable salts thereof, characterized in that a divalent metal salt of a sulfonated p-amino phenylalanine derivative is prepared, and a peptide chain is elongated by use of said divalent metal salt.

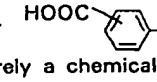
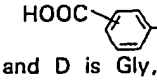
According to still further an aspect of the present invention, there is provided an accelerator for pancreatic function which contains a novel peptide represented by the following formula

$R_1$-A-B-C-D-Trp-Met-Y (1)

wherein; $R_1$ denotes pGlu, X-$R_2$-CO-, X being carboxyl or amino and $R_2$ being lower alkylene of 1-6 carbon atoms, or  ; A denotes Asp, Ala, or merely a chemical bond; B denotes Tyr($SO_3H$) or Phe($NHSO_3H$); C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes $NH_2$, Asp-$NH_2$, or Asp-Phe-$NH_2$; with the proviso that Y is $NH_2$ or Asp-$NH_2$ when $R_1$ is pGlu, HOOC-$R_2$-CO-, or  , B is Tyr($SO_3H$), C is Met, and D is Gly, and pharmacologically acceptable salts thereof, as an effective component.

According to still further an aspect of the present invention, there is provided a reagent for pancreatic function tests or a contrast medium for gallbladder which contains a novel peptide represented by the following formula

$R_1$-A-B-C-D-Trp-Met-Y (1)

wherein; $R_1$ denotes pGlu, X-$R_2$-CO-, X being carboxyl or amino and $R_2$ being lower alkylene of 1-6 carbon atoms, or  ; A denotes Asp, Ala, or merely a chemical bond; B denotes Tyr($SO_3H$) or Phe($NHSO_3H$); C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes $NH_2$, Asp-$NH_2$, or Asp-Phe-$NH_2$; with the proviso that Y is $NH_2$ or Asp-$NH_2$ when $R_1$ is pGlu, HOOC-$R_2$-CO-, or  , B is Tyr($SO_3H$), C is Met, and D is Gly, and pharmacologically acceptable salts thereof as an effective component.

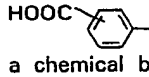
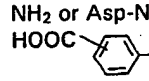
*Brief description of the drawings*

Figs. 1 and 2 show pancreatic secretion accelerating activities of various peptides of the present invention compared with that of CCK-8. Figs. 3 and 4 show whole protein secretion accelerating activities of those peptides compared with that of CCK-8.

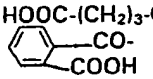
*Detailed description of the preferred embodiments*

The peptide of the present invention is novel and represented by the general formula

$R_1$-A-B-C-D-Trp-Met-Y (1)

wherein; $R_1$ denotes pGlu, X-$R_2$-CO-, X being carboxyl or amino and $R_2$ being lower alkylene, or  ; A denotes Asp, Ala, or merely a chemical bond (this means that $R_1$ combines directly with B); B denotes Tyr ($SO_3H$) or Phe($NHSO_3H$); C denotes Met or merely a chemical bond (this means that B combines directly with D); D denotes Gly, D-Ala, or D-Trp; and Y denotes $NH_2$, Asp-$NH_2$, or Asp-Phe-$NH_2$; with the proviso that Y is $NH_2$ or Asp-$NH_2$ when $R_1$ is pGlu, HOOC-$R_2$-CO- or  -CO-, B is Tyr($SO_3H$), C is Met, and D is Gly.

In the present specification, the abbreviations of amino acids, peptides, protective groups, active groups, and other compounds and groups are in accordance with those defined in IUPAC or IUB or with those customarily used in the art. Examples of the abbreviations are given below. Unless otherwise specified, compounds such as amino acids, when they can involve optical isomers, refer to L-isomers thereof.

| | |
|---|---|
| Asp | Aspartic acid residue |
| Ala | Alanine residue |
| β-Ala | β-Alanine residue |
| Leu | Leucine residue |
| Gly | Glycine residue |
| Met | Methionine residue |
| pGlu | Pyroglutamic acid residue |
| Phe | Phenylalanine residue |
| Trp | Tryptophan residue |
| Tyr | Tyrosine residue |
| Bz | Benzyl group |
| Boc | tert-Butyloxycarbonyl group |
| Z | Benzyloxycarbonyl group |
| OSu | Succinimidoxy group |
| Suc | HOOC-$(CH_2)_2$-CO- |
| Glt | HOOC-$(CH_2)_3$-CO- |
| Pht |  |
| DMF | Dimethylformamide |
| THF | Tetrahydrofuran |

The peptide of the general formula (1) can be prepared by common methods applied for the synthesis of peptides, for example, methods described in «The Peptides», Vol. 1 (1966) (by Schroder and Luhke, published by Academic Press, New York, U.S.A.) and «Peptide Synthesis» (1975) (by Izumiya et al., published by Maruzen Co., Ltd.). These processes, applicable in the invention, include the azide method, acid chloride method, acid anhydride method, mixed anhydride method, DCC

method, active ester method (p-nitrophenyl) method, N-hydroxysuccimide ester method, cyanomethyl ester method, or the like, the method employing Woodward's reagent K, carbodiimidazole method, oxidation-reduction method, DCC-additive method (HONB, HOBT, HOSu), and solid phase method, etc.

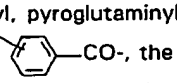
Usually, the peptide of the general formula (1) is prepared according to the above-mentioned general method for the synthesis of polypeptides, for instance, the so-called stepwise process which comprises successive condensations of individual amino acids with a terminal amino acid or the process which comprises coupling reactions of a few fragments to complete the intended polypeptide. More particularly, the present peptide can be prepared by the condensation of a reactive-carboxyl-containing compound with a reactive-amino-containing compound in the usual way, the former compound corresponding to one of the two moieties into which the peptide can be divided by cutting its main chain at an arbitrary position and the latter compound corresponding to the other moiety. When the condensation product has protective groups, the preparation is possible through eliminating the protective groups in the usual way. Aspartic acid, when used in the reaction process for synthesizing the peptide of the formula (1) is desired in most cases to be protected previously. In the final step of the reaction process, all the protective groups are usually eliminated from the peptide in which one or more of the constituent amino acid residues are protected.

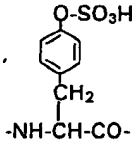
In the reaction process for synthesizing the peptide of the formula (1), the functional groups which should not participate in the reaction are also protected by common protective groups, which are eliminated after completion of the reaction. Moreover, the functional groups to participate in the reaction are activated in general.

The above reaction methods are known and reagents used therein can be suitably selected from known compounds.

Protective groups for the amino group include, for example, carbobenzoxy (hereinafter abbreviated as Z), tert-butyloxycarbonyl (hereinafter abbreviated as Boc), tert-amyloxycarbonyl, isobornyloxycarbonyl, p-methoxybenzyloxycarbonyl, 2-chloro-benzyloxycarbonyl, adamantyloxycarbonyl, trifluoroacetyl, phthalyl, formyl, o-nitrophenylsulphenyl, and diphenylphosphinothioyl, etc. Protective groups for the carboxyl group include, for example, alkyl esters (e.g. esters of methyl, ethyl, propyl, butyl, and tert-butyl), benzyl ester, p-nitrobenzyl ester, p-chlorobenzyl ester, benzhydryl ester, carbobenzoxyhydrazide, tert-butyloxycarbonylhydrazide, and tritylhydrazide, etc.

Activated derivatives of the carboxyl group include, for example, acid chloride, acid anhydride or mixed anhydride, azides, and activated esters (esters of pentachlorophenol, p-nitrophenol, N-hydroxysuccinimide, N-hydroxybenzotriazole, and N-hydroxy-5-norbornene-2,3-dicarboxyimide, and the like). Sometimes the peptide-linkage forming reaction can be carried out in the presence of a condensing agent such as a carbodiimide reagent (e.g., dicyclohexylcarbodiimide or carbodiimidazole) or tetraethyl pyrophosphate, etc.

In the above general formula (1), when $R_1$ is X-$R_2$-CO- (X denotes carboxyl or amino and $R_2$ denotes lower alkylene), $R_2$ denotes a $C_1$-$C_6$ alkylene. In consequence, $R_1$ in this case denotes succinyl, glutaryl, maleyl, phthalyl, glycyl, β-alanyl, γ-aminobutyryl, pyroglutaminyl, or the like. When $R_1$ is  , the carboxyl group can be attached to the benzene ring at any of the o-, m-, and p-positions.

The peptide of the formula (1) wherein B is Tyr($SO_3H$), i.e.,  , is favorably prepared in the following way:

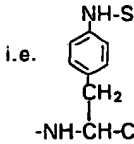
A peptide of the general formula

$R_1$-A-Tyr-C-D-Trp-Met-Y (2)

[$R_1$, A, C, D, and Y are the same as defined in the formula (1)] which is protected if necessary by masking its active group such as amino group with a suitable protective group is synthesized, and sulfate-esterified to convert the Tyr residue into Tyr($SO_3H$).

For the peptide preparation specially requiring the elimination of a protective group after sulfate-esterification, the process disclosed in USP No. 4,330,466 is best suited in respect to the stabilization of the sulfate ester and the prevention of side reaction. This process comprises concentrating the sulfate-esterification reaction mixture and adding a solvent such as methanol, butanol, ethanol, dimethylformamide, or water and an aqueous solution of water-soluble salt of a divalent metal such as calcium or zinc to the concentrate to stabilize it, followed by eliminating the protective group.

The way of the sulfate-esterification is already known. For instance, the esterification is carried out by dissolving a peptide of the formula (2) in an inert solvent such as dimethylformamide or pyridine and adding a pyridine-anhydrous sulfuric acid complex in an amount of about 10 times the amount of the peptide. The reaction is preferably conducted first at a low temperature and then at room temperature, for 15-20 hours.

A peptide of the formula (1) wherein B is Phe($NHSO_3H$), i.e.  , is favorably prepared in the following way:

A p-aminophenylalanine derivative of which the α-amino group is protected, e.g. Boc-Phe($NH_2$)-OH, is reacted with a sulfonating reagent, e.g. a pyridine-sulfuric anhydride complex to sulfonate the p-amino group, the Boc-Phe($NHSO_3^-$)-OH is converted into a divalent metal salt by reacting with a salt of

divalent metal such as calcium in the same manner as mentioned above, the salt of Boc-Phe($NHSO_3^-$)-OH is condensed with a peptide fragment of the general formula

C-D-Trp-Met-Y (3)

[C, D, and Y are the same as defined in the formula (1)] by a suitable method, e.g. the active ester process, DCC process, or the like, the resulting metal salt of the peptide of the general formula

Boc-Phe($NHSO_3^-$)-C-D-Trp-Met-Y (4)

[C, D, and Y are the same as defined in the formula (1)] is treated with trifluoroacetic acid or with some other reagent to aliminate the protective group, and the resulting peptide is further condensed with the necessary acyl group and amino acid (corresponding to $R_1$ and A).

A peptide of the formula (1) wherein B is Phe($NHSO_3H$) is also prepared in the following way:

A peptide represented by the general formula

$R_1$-A-Phe($NH_2$)-C-D-Trp-Met-Y (5)

[$R_1$, A, C, D, and Y are the same as defined in the formula (1)] which is protected if necessary by masking its active group with a suitable protective group is synthesized and sulfonated to convert the Phe($NH_2$) residue into Phe($NHSO_3H$).

The thus obtained peptide of the invention, represented by the formula (1), can be desalted and purified according to common methods, for example, ion-exchange chromatography employing DEAE-Cellulose or the like, partition chromatography employing Sephadex LH-20 or Sephadex G-25, normal phase chromatography employing silica gel or the like, reversed phase chromatography employing ODS-silica gel or the like, and high performance liquid chromatography (HPLC).

Typical examples of the peptide represented by the formula (1) are as follows:

Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$
Suc-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$
Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-$NH_2$
Suc-Tyr($SO_3H$)-Met-D-Ala-Trp-Met-Asp-Phe-$NH_2$
Suc-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$
Suc-Phe($NHSO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$
Gly-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$
β-Ala-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$
pGlu-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$
Glt-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$
Pht-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$
Glt-Tyr($SO_3H$)-Met-D-Ala-Trp-Met-Asp-Phe-$NH_2$
Glt-Phe($NHSO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$
Glt-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$
Glt-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-$NH_2$
Gly-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$
β-Ala-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$
Glt-Ala-Tyr($SO_3H$)-Gly-Trp-Met-Asp-Phe-$NH_2$
pGlu-Ala-Tyr($SO_3H$)-Gly-Trp-Met-Asp-Phe-$NH_2$

The peptide of the formula (1) can be made up into pharmaceutically acceptable salts, e.g. salts of alkali metals such as sodium and potassium, salts of alkaline earth metals such as calcium, and salts of triethylamine and ammonium.

The peptide of the invention has physiological actions similar to that of «CCK-8» of the following formula

Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$.

It has been found that peptides of the present invention have properties which are not found in CCK-8, in respect to the contraction of ginea pig gallbladder, enhancement of rat pancreatic juice secretion, and stimulation of pancreatic protein secretion, among the previously mentioned physiological actions of CCK-8.

A series of peptides of the invention having D-Ala or D-Trp as D of the formula (1) exhibits almost no action on the gallbladder while having markedly strong action on the pancreas. Moreover, a series of peptides of the invention having X-$R_2$-CO- as $R_1$ wherein X is amino is lower in the activity on the pancreas though equal or higher in the activity on the gallbladder, than CCK-8. Certain peptides of the invention are 2-6 times and 50-260 times as effective as tetragastrin in the acceleration of gastric secretion and the pancreatic external secretion, respectively.

Accordingly, certain peptides of the formula (1) according to the present invention are useful as medicines for a specific organ, (for example, the accelerator for the pancreatic function) and as specific reagents for laboratory tests (for example, the pancreatic function testing reagent and a contrast medium for gallbladder) and expected also to be gastrin-like peptides. Furthermore, other peptides of the invention can be expected antagonists of CCK-8 or gastrin.

The process for producing peptides of the invention is illustrated referring to the following examples; however, the invention is not limited to these examples. Abbreviations in the examples are as defined already.

*Example 1*

*Preparation of Suc-Tyr($SO_3H$)-Met-Gly-Trp--Met-$NH_2$ (Compound I)*

(1) Synthesis of Boc-Trp-Met-$NH_2$

5.78 g (0.039 mole) of H-Met-$NH_2$ was dissolved in 50 mℓ of DMF, then 5.46 mℓ of triethylamine and further 18.70 g (0.047 mole) of Boc-Trp-OSu were added under cooling with ice, and the mixture was stirred overnight at room temperature. The reaction mixture was concentrated in vacuo, and extracted with ethyl acetate. The extract was washed with 1N citric acid, saturated saline solution, saturated sodium bicarbonate solution, and saturated saline solution in series. The resulting organic layer was dried over anhydrous sodium sulfate, distilled in vacuo to remove the solvent, and solidified with hexane. The solid was recrystallized from ethyl acetate-hexane, giving 12.45 g of Boc-Trp-Met--$NH_2$; yield 73.5%, m.p. 155-157°C.

$[\alpha]_D^{24}$ = —3.6° (C = 1, methanol).

Anal. Calcd. (%) for $C_{21}H_{30}N_4O_4S$: C, 50.04; H, 6.96; N, 12.86. Found (%): C, 50.14; H, 7.18; N, 12.68.

(2) Synthesis of Boc-Met-Gly-Trp-Met-$NH_2$

11.73 g (0.027 mole) of the dipeptide obtained in the above (1) was dissolved by adding 20 mℓ of trifluoroacetic acid containing 0.4 mℓ of ethandithiol and reacted at room temperature for 30 minutes. Then, a precipitate was formed by adding 200 mℓ of anhydrous ether to the reaction mixture and was filtered and dried. On the other hand, 12.98 g of Boc-Met-Gly-$NHNH_2$ was dissolved in 100 mℓ of DMF, and after cooling with dry ice-ethanol to —20°C or lower, was converted into azide by adding 20.25 mℓ of a 6N HCℓ-dioxane mixture and 5.44 mℓ of isoamyl nitrite. The reaction mixture was neutralized by further adding 17.01 mℓ of triethylamine, and admixed with a solution of the above deprotected dipeptide in 100 mℓ of DMF. The mixture was stirred at —20°C for 2 hours and at 4°C for 17 hours. The resulting reaction mixture was extracted with ethyl acetate similarly to the above (1), the solvent was distilled off from the extract, and the residue was solidified with ether. The solid was filtered off, dried, and recrystallized from methanol-ether, giving 12.60 g of Boc-Met-Gly-Trp-Met-$NH_2$; yield 74.9%, m.p. 200-202°C.

$[\alpha]_D^{24}$ = —24.3° (C = 1, DMF).

Anal. Calcd. (%) for $C_{28}H_{42}N_6O_6S_2$: C, 54.00; H, 6.80; N, 13.49. Found (%): C, 54.21; H, 6.81; N, 13.31.

(3) Synthesis of Boc-Tyr-Met-Gly-Trp-Met-$NH_2$

The protective group was eliminated from 6.85 g (0.011 mole) of the tetrapeptide obtained in the above (2), by using trifluoroacetic acid in the same manner as in above (2). The resulting tetrapeptide was dissolved in 50 mℓ of DMF, and 1.54 mℓ of triethylamine and 8.32 g (0.022 mole) of Boc-Tyr-OSu were added under cooling with ice. After stirring overnight at room temperature, the reaction mixture was concentrated in vacuo and extracted with ethyl acetate. The extract was concentrated, and ether was added to deposit a solid, which was then filtered, dried, and recrystallized from methanol-ethyl acetate, giving 5.46 g of Boc-Tyr-Met-Gly-Trp-Met-$NH_2$; yield 63.1%, m.p. 195-198°C.

$[\alpha]_D^{24}$ = —21.8° (C = 1, DMF).

Anal. Calcd. (%) for $C_{39}H_{51}N_7O_8S_2$: C, 56.54; H, 6.54; N, 12.47. Found (%): C, 56.67; H, 6.63; N, 12.25.

(4) Synthesis of Suc-Tyr-Met-Gly-Trp-Met-$NH_2$

The protective group was eliminated from 3.45 g (0.0044 mole) of the pentapeptide obtained in the above (3), by the same treatment as applied in the above (2). The treated pentapeptide was dissolved in DMF, and 1.23 mℓ of triethylamine and 0.88 g of succinic anhydride were added under cooling with ice. The mixture was stirred at room temperature for 1 day. The solvent was distilled off, 1N citric acid was added to the residue, and the resulting precipitate was filtered off, washed with water, and recrystallized from methanol-ethyl acetate, giving 2.52 g of Suc-Tyr-Met-Gly-Trp-Met-$NH_2$; yield 72.8%, m.p. 125-127°C.

$[\alpha]_D^{24}$ = —28.5° (C = 1, DMF).

Anal. Calcd. (%) for $C_{36}H_{47}N_7O_9S_2$: C, 55.02; H, 6.03; N, 12.48. Found (%): C, 54.91; H, 6.20; N, 12.42.

(5) Synthesis of Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$

1.57 g (0.002 mole) of the acylated peptide obtained in the above (4) was dissolved in a mixture of 20 mℓ of DMF and 2 mℓ of pyridine, and 3.18 g (0.020 mole) of a pyridine-sulfuric anhydride complex was added under cooling with ice. The mixture was stirred at 0°C for 30 minutes and at room temperature for 20 hours. The reaction mixture was concentrated in vacuo, 50 mℓ of 0.05 M aqueous ammonium carbonate solution was added, and the pH was adjusted to 8.5 by addition of aqueous ammonia. The solution was purified by chromatography on a DEAE-Cellulose column (5 × 15 cm), wherein the adsorption and washing were carried out by using 1.5 ℓ of an 0.05 M buffer solution of ammonium carbonate-ammonium bicarbonate (pH 8.5) and eluted with 2.0 ℓ of the same but 0.2 M buffer solution (pH 8.5). Eluted fractions were measured for ultraviolet absorbance in a wavelength of 278 nm, and thereby the fractions containing the intended product were collected, concentrated, and freeze-dried, giving 0.85 g of Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$ (Compound 1); yield 50.0%.

$[\alpha]_D^{25}$ = —15.8° (C = 0.5, 1N ammonia).

Anal. Calcd. (%) for $C_{36}H_{47}N_7O_{12}S_3 \cdot NH_3 \cdot H_2O$: C, 47.99; H, 5.82; N, 12.44. Found (%): C, 47.94; H, 5.59; N, 12.00.

Amino acid analysis by acidolysis:

Gly 1.00 (1), Met 1.98 (2), Tyr 1.01 (1)

Infrared absorption spectroscopy of the product indicates a strong absorption peak characteristic of sulfate ester at 1050 $cm^{-1}$.

*Example 2*

*Preparation of Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-$NH_2$ (Compound II)*

(1) Synthesis of Boc-Met-Asp-$NH_2$

12.47 g (0.035 mole) of Z-Asp(OBz)-$NH_2$ (m.p. 118-120°C. $[\alpha]_D^{24}$ = —2.6° (C = 1, methanol). Anal. Calcd. (%) for $C_{18}H_{20}N_2O_5$: C, 64.04; H, 5.66; N, 7.86. Found (%): C, 64.04; H, 5.73; N, 7.73) was dissolved in a mixture of 300 mℓ of methanol and 35 mℓ of 1N HCℓ and subjected to contact reduction with hydrogen (at room temperature for 6 hours) in the presence of 1.75 g of a 10% Pd-carbon catalyst. The catalyst was then filtered off, the solvent was distilled off, and the residue was dried in vacuo. On the other hand, 9.36 g (0.035 mole) of Boc-Met-OH was dissolved in 50 mℓ of THF, the solution was cooled to —20°C, and 3.85 mℓ of N-methylmorpholine and 4.62 mℓ of isobutyl chloroformate were added. The resulting mixed anhydride was admixed with a solution of the above obtained H-Asp-$NH_2$ in 50 mℓ of DMF (containing 4.90 mℓ of triethylamine). The mixture was stirred at 0°C for

methanol-ether giving 19.90 g of Boc-Met-D--Ala-Trp-Met-Asp-Phe-$NH_2$; yield 69.2%, m.p. 199-202°C.

$[\alpha]_D^{24}$ = —30.0° (C = 1, DMF).

Anal. Calcd. (%) for $C_{42}H_{58}N_8O_{10}S_2$: C, 56.11; H, 6.50; N, 12.46. Found (%): C, 56.00; H, 6.93; N, 12.46.

(3) Synthesis of Boc-Tyr-Met-D-Ala-Trp-Met--Asp-Phe-$NH_2$

In the same manner as in (3) of Example 1, 9.90 g (0.011 mole) of the hexapeptide obtained in the preceding (2) was condensed with 8.23 g of Boc--Tyr-OSu. The product was recrystallized from methanol-ethyl acetate, giving 8.36 g of Boc-Tyr--Met-D-Ala-Trp-Met-Asp-Phe-$NH_2$; yield 71.5%, m.p. 202-205°C.

$[\alpha]_D^{24}$ = —25.8° (C = 1, DMF).

Anal. Calcd. (%) for $C_{51}H_{69}N_9O_{13}S_2 \cdot H_2O$: C, 56.70; H, 6.44; N, 11.67. Found (%): C, 57.00; H, 6.54; N, 11.22.

(4) Synthesis of Suc-Tyr-Met-D-Ala-Trp-Met--Asp-Phe-$NH_2$

In the same manner as in (4) of Example 1, the protective group was eliminated from 4.02 g (0.0038 mole) of the heptapeptide obtained in the preceding (3). The deprotected heptapeptide was reacted with 0.76 g (0.0076 mole) of succinic anhydride. The product was recrystallized from methanol-ethyl acetate, giving 3.85 g of Suc-Tyr-Met-D-Ala-Trp--Met-Asp-Phe-$NH_2$; yield 95.8%, m.p. 200-203°C.

$[\alpha]_D^{24}$ = —29.3° (C = 1, DMF).

Anal. Calcd. (%) for $C_{50}H_{63}N_9O_{13}S_2$: C, 56.54; H, 5.98; N, 11.87. Found (%): C, 56.26; H, 6.36; N, 12.05.

(5) Synthesis of Suc-Tyr($SO_3H$)-Met-D-Ala-Trp--Met-Asp-Phe-$NH_2$

In the same manner as in (6) of Example 2, 2.12 g (0.002 mole) of the Suc-Tyr-Met-D-Ala-Trp-Met--Asp-Phe-$NH_2$ obtained in the preceding (4) was sulfated and purified by chromatography on a DEAE-Cellulose column (5 × 15 cm). Thus, 1.25 g of Suc-Tyr($SO_3H$)-Met-D-Ala-Trp-Met-Asp-Phe-$NH_2$ (Compound III) was obtained as a lyophilized product; yield 54.9%.

Anal. Calcd. (%) for $C_{50}H_{63}N_9O_{10}S_3 \cdot NH_3 \cdot 3H_2O$: C, 49.50; H, 5.98; N, 11.54. Found (%): C, 49.67; H, 5.71; N, 11.52.

Amino acid analysis by acidolysis:

Asp 1.05 (1), Gly 0.99 (1), Met 2.00 (2), Tyr 1.01 (1), Phe 1.01 (1)

Infrared absorption spectrum: 1050 $cm^{-1}$ (sulfate ester).

*Example 4*

*Preparation of Suc-Tyr($SO_3H$)-Met-D-Trp-Trp--Met-Asp-Phe-$NH_2$ (Compound IV)*

(1) Synthesis of Boc-D-Trp-Trp-Met-Asp-Phe--$NH_2$

In the same manner as in (1) of Example 1, 10.45 g (0.015 mole) of Boc-Trp-Met-Asp-Phe-$NH_2$ [cf. (1) of Example 3] was condensed with 9.00 g (0.022 mole) of Boc-Trp-OSu. The resulting reaction liquid was concentrated, and a precipitate was formed by adding 1N citric acid and washed with water. Recrystallization thereof from methanol-water gave 8.09 g of Boc-D-Trp-Trp-Met-Asp-Phe-$NH_2$; yield 61.1%, m.p. 225-227°C.

$[\alpha]_D^{24}$ = —24.0° (C = 1, DMF).

Anal. Calcd. (%) for $C_{45}H_{54}N_8O_9S$: C, 61.21; H, 6.16; N, 12.69. Found (%): C, 61.44; H, 6.30; N, 12.55.

(2) Synthesis of Boc-Met-D-Trp-Trp-Met-Asp--Phe-$NH_2$

In the same manner as in (2) of Example 3, 7.94 g (0.009 mole) of the pentapeptide obtained in the preceding (1) was condensed with 3.37 g of Boc--Met-OH. The product was recrystallized from methanol-water, giving 7.00 g of Boc-Met-D--Trp-Trp-Met-Asp-Phe-$NH_2$; yield 76.7%, m.p. 195-197°C.

$[\alpha]_D^{24}$ = —28.1° (C = 1, DMF).

Anal. Calcd. (%) for $C_{50}H_{63}N_9O_{10}S_2$: C, 59.21; H, 6.26; N, 12.43. Found (%): C, 59.08; H, 6.37; N, 12.24.

(3) Synthesis of Boc-Tyr-Met-D-Trp-Trp-Met--Asp-Phe-$NH_2$

In the same manner as in (3) of Example 1, 3.55 g (0.0035 mole) of the hexapeptide obtained in the preceding (2) was freed of the protective group and then condensed with 2.65 g of Boc-Tyr-OSu. The product was recrystallized from methanol-water, giving 3.51 g of Boc-Tyr-Met-D-Trp-Trp-Met-Asp--Phe-$NH_2$; yield 85.2%, m.p. 204-206°C.

$[\alpha]_D^{24}$ = —26.3° (C = 1, DMF).

Anal. Calcd. (%) for $C_{59}H_{92}N_{10}O_{12}S_2$: C, 60.19; H, 6.16; N, 11.90. Found (%): C, 59.93; H, 6.28; N, 11.35.

(4) Synthesis of Suc-Tyr-Met-D-Trp-Trp-Met--Asp-Phe-$NH_2$

In the same manner as in (4) of Example 1, 3.41 g (0.0029 mole) of the heptapeptide obtained in the preceding (3) was deprotected, and then reacted with 0.58 g of succinic anhydride. The product was recrystallized from methanol-ether, giving 3.17 g of Suc-Tyr-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$; yield 92.8%, m.p. 198-201°C.

$[\alpha]_D^{24}$ = —31.1° (C = 1, DMF).

Anal. Calcd. (%) for $C_{58}H_{68}N_{10}O_{13}S_2$: C, 59.17; H, 5.82; N, 11.90. Found (%): C, 59.40; H, 6.06; N, 11.75.

(5) Synthesis of Suc-Tyr($SO_3H$)-Met-D-Trp-Trp--Met-Asp-Phe-$NH_2$

In the same manner as in (5) of Example 1, 2.36 g (0.002 mole) of the acylated peptide obtained in the preceding (4) was sulfated with 3.18 g of a pyridine-sulfuric anhydride complex. The product was purified by chromatography on a DEAE-Cellulose column (5 × 15 cm), wherein 2 ℓ of a 30% methanol-0.5 M ammonium carbonate buffer solution (pH 8.5) was used for elution. Thus, 1.28 g of Suc-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$

(Compound IV) was obtained as a lyophilized product; yield 50.9%.

$[\alpha]_D^{24}$ = —15.0° (C = 1, 1N ammonia).

Anal. Calcd. (%) for $C_{58}H_{66}N_{10}O_{16}S_3 \cdot NH_3 \cdot 3H_2O$: C, 52.44; H, 5.84; N, 11.60. Found (%): C, 52.21; H, 5.71; N, 11.91.

Amino acid analysis by acidolysis:
Asp 1.03 (1), Met 1.98 (2), Tyr 1.00 (1), Phe 0.99 (1).

*Example 5*
*Preparation of Glt-Tyr($SO_3H$)-Met-D-Trp-Trp--Met-Asp-Phe-$NH_2$ (Compound V)*

Similarly to (4) of Example 4, 1.77 g (0.0015 mole) of the Boc-Tyr-Met-D-Trp-Trp-Met-Asp-Phe--$NH_2$ obtained in (3) of Example 4 was deprotected and reacted with 0.342 g of glutaric anhydride. The product was recrystallized from methanol-ether, giving 1.50 g of Glt-Tyr-Met-D-Trp-Trp-Met-Asp--Phe-$NH_2$: yield 83.9%, m.p. 208-210°C.

$[\alpha]_D^{24}$ = —25.7° (C = 1, DMF).

Anal. Calcd. (%) for $C_{59}H_{70}N_{10}O_{13}S_2 \cdot H_2O$: C, 58.60; H, 6.00; N, 11.58. Found (%): C, 58.49; H, 6.08; N, 11.52.

Similarly to (5) of Example 4, 1.30 g of the above peptide was sulfated and purified by chromatography on a DEAE-Cellulose column (4×15 cm). Thus, 683 mg of Glt-Tyr($SO_3H$)-Met-D-Trp-Trp--Met-Asp-Phe-$NH_2$ (Compound V) was obtained as a lyophilized product; yield 49.3%.

Anal. Calcd. (%) for $C_{59}H_{70}N_{10}O_{16}S_3 \cdot NH_3 \cdot 4H_2O$: C, 52.09; H, 6.00; N, 11.32. Found (%): C, 51.92; H, 6.11; N, 11.33.

Amino acid analysis by acidolysis:
Asp 1.05 (1), Met 1.94 (2), Tyr 1.03 (1), Phe 0.98 (1).

*Example 6*
*Preparation of Pht-Tyr($SO_3H$)-Met-D-Trp-Trp--Met-Asp-Phe-$NH_2$*

Similarly to (4) of Example 4, 1.77 g (0.0015 mole) of the Boc-Tyr-Met-D-Trp-Trp-Met-Asp-Phe--$NH_2$ obtained in (3) of Example 4 was deprotected and condensed with 0.444 g of phthalic anhydride. The resulting product was recrystallized from methanol-ether, giving 1.79 g of Pht-Tyr-Met--D-Trp-Trp-Met-Asp-Phe-$NH_2$; yield 97.4%, m.p. 180-183°C.

$[\alpha]_D^{24}$ = —31.4° (C = 1, DMF).

Anal. Calcd. (%) for $C_{62}H_{68}N_{10}O_{13}S_2 \cdot H_2O$: C, 59.89; H, 5.67; N, 11.26. Found (%): C, 59.55; H, 5.97; N, 11.45.

Similarly to (5) of Example 4, 1.59 g of the above peptide was sulfated and purified by chromatography on a DEAE-Cellulose column (4×15 cm). Thus, 638 mg of Pht-Tyr($SO_3H$)-Met-D-Trp-Trp--Met-Asp-Phe-$NH_2$ (Compound VI) was obtained as a lyophilized product; yield 37.6%.

$[\alpha]_D^{24}$ = —18.3° (C = 1, 1N ammonia).

Anal. Calcd. (%) for $C_{62}H_{68}N_{10}O_{16}S_3 \cdot NH_3 \cdot 4H_2O$: C, 53.40; H, 5.71; N, 11.05. Found (%): C, 53.61; H, 5.65; N, 10.59.

Amino acid analysis by acidolysis:
Asp 1.06 (1), Met 1.98 (2), Tyr 0.96 (1), Phe 0.99 (1).

*Example 7*
*Preparation of Suc-Phe($NHSO_3H$)-Met-Gly-Trp--Met-Asp-Phe-$NH_2$ (Compound VII)*

(1) Synthesis of Boc-Phe($NH_2$)-OH

3.27 g (0.014 mole) of H-Phe($NO_2$)-OH and 3.26 mℓ of triethylamine were dissolved in 50 mℓ of water, and 50 mℓ of a dioxane solution of 4.10 g (0.017 mole) of a Boc-S reagent, e.g. t-butyl S-4,6--dimethyl pyrimidin-2-yl thiolcarbonate (made by Kokusan Kagaku Co., Ltd.) was added under cooling with ice. After one day stirring at room temperature, the reaction mixture was diluted with 50 mℓ of water and washed with ethyl acetate. The aqueous layer, cooled with ice, was acidified to pH 2 by adding 6N HCℓ. The mixture was extracted with ethyl acetate, and the extract was washed with 1N HCℓ and then saturated saline solution, and dried over anhydrous sodium sulfate. The ethyl acetate was distilled off in vacuo, hexane was added to the residue, and the solid deposit was filtered and dried. Recrystallization thereof from ethyl acetate-hexane gave 4.04 g of Boc-Phe($NO_2$)-OH; yield 84.0%, m.p. 110-112°C.

$[\alpha]_D^{24}$ = +7.7° (C = 1, methanol).

Anal. Calcd. (%) for $C_{14}H_{18}N_2O_6$: C, 54.19; H, 5.85; N, 9.03. Found (%): C, 54.13; H, 5.77; N, 9.10.

3.93 g (0.013 mole) of the above obtained Boc--Phe($NO_2$)-OH was dissolved in 50 mℓ of methanol and hydrogenated over 1.3 g of a 5% Pd-carbon catalyst at room temperature for 8 hours. Then, the catalyst was filtered off, and the filtrate was concentrated in vacuo. Hexane was added to the residual to form a precipitate, which was then recrystallized from ethyl acetate-ether, giving 2.39 g of Boc-Phe($NH_2$)-OH; yield 67.1%, m.p. 126-128°C.

$[\alpha]_D^{24}$ = +26.6° (C = 1, methanol).

Anal. Calcd. (%) for $C_{14}H_{20}N_2O_4$: C, 59.99; H, 7.19; N, 9.99. Found (%): C, 59.79; H, 7.15; N, 9.85.

(2) Synthesis of Boc-Phe($NHSO_3^-$)-$O^-$·calcium salt

1.26 g (0.0045 mole) of the Boc-Phe($NH_2$)-OH obtained in the preceding (1) was dissolved in a mixture of 20 mℓ of DMF and 2 mℓ of pyridine, and 3.58 g of a pyridine-sulfuric anhydride complex was added under cooling with ice. The mixture was stirred at 0°C for 30 minutes and then at room temperature for 20 hours. The resulting reaction mixture was concentrated in vacuo, 30 mℓ of ice-cold water was added to the residue to dissolve it, and 27 mℓ of 1 M aqueous calcium acetate solution was added immediately. The resulting calcium sulfate precipitate was removed by centrifugation, carbon dioxide gas was blown into the supernatant, and the resulting calcium carbonate precipitate was removed by centrifugation. The resulting supernatant was concentrated in vacuo. The residue was

recrystallized from ethanol-ether, giving Boc-Phe-($NHSO_3^-$)-$O^-\cdot Ca^{2+}$.

(3) Synthesis of Boc-Phe($NHSO_3H$)-Met-Gly--Trp-Met-Asp-Phe-$NH_2$

1.71 g (0.0045 mole) of the sulfonated amino acid obtained in the preceding (2) was dissolved in 20 mℓ of DMF, then 0.57 g of N-hydroxysuccinimide and 0.93 g of dicyclohexylcarbodiimide were added, and the mixture was stirred overnight at 4°C. The precipitated urea compound was filtered off, the filtrate was concentrated in vacuo, ether was added to the residue, and the resulting precipitate was filtered and dried, giving Boc-Phe($NHSO_3^-$)--OSu·1/2 Ca salt.

On the other hand, 2.66 g (0.003 mole) of Boc--Met-Gly-Trp-Met-Asp-Phe-$NH_2$ [m.p. 195-197°C. $[\alpha]_D^{24}$ = —30.0° (C = 1, DMF). Anal. Calcd. (%) for $C_{41}H_{56}N_8O_{12}S_2$: C, 55.64; H, 6.38; N, 12.66. Found (%): C, 55.85; H, 6.55; N, 12.54. cf. M.A. Ondett et al., Journal of the Americal Chemical Society, 92, 195 (1970)] was dissolved in 6 mℓ of trifluoroacetic acid containing 0.2 mℓ of ethandithiol, and the solution was allowed to stand at room temperature for 30 minutes. Then, 70 mℓ of anhydrous ether was added to the reaction mixture, and the resulting precipitate was filtered and dried. The deprotected peptide was dissolved in DMF, and 0.42 mℓ of triethylamine was added to the solution cooled with ice.

To the resulting solution was added to the above obtained active ester [Boc-Phe($NHSO_3^-$)-OSu·1/2 Ca salt], and the mixture was stirred overnight at room temperature. The reaction mixture was then concentrated in vacuo, and a precipitate was formed by adding ethyl acetate to the residue. The precipitate was filtered and dried, giving 2.20 g of Boc-Phe-($NHSO_3^-$)-Met-Gly-Trp-Met-Asp-Phe--$NH_2$·Ca salt.

(4) Synthesis of Suc-Phe($NHSO_3H$)-Met-Gly--Trp-Met-Asp-Phe-$NH_2$

The heptapeptide calcium salt obtained in the preceding (3) was dissolved in 10 mℓ of trifluoroacetic acid containing 0.2 mℓ of ethandithiol, and the solution was allowed to stand at 0°C for 30 minutes. To the reaction mixture was added 100 mℓ of anhydrous ether, and the resulting precipitate was filtered and dried. This deprotected peptide was dissolved in 30 mℓ of DMF, and reacted with 1.3 g of succinic anhydride in the same manner as in (4) of Example 1. The resulting reaction mixture was concentrated in vacuo, and purified in the same manner as in (5) of Example 1 by chromatography on a DEAE-Cellulose column (5 x 15 cm). Thus, 460 mg of Suc-Phe($NHSO_3H$)-Met-Gly-Trp-Met-Asp--Phe-$NH_2$ (Compound VII) was obtained as a lyophilized product.

$[\alpha]_D^{25}$ = —17.0° (C = 0.5, 1N ammonia).

Anal. Calcd. (%) for $C_{49}H_{62}N_{10}O_{15}S_2\cdot 3H_2O$: C, 49.82; H, 5.80; N, 11.86. Found (%): C, 49.63; H, 5.61; N, 11.96.

Amino acid analysis by acidolysis:

Asp 1.08 (1), Gly 1.05 (1), Met 2.04 (2), Phe 1.00 (1), Phe($NH_2$) 0.84 (1).

*Example 8*

*Preparation of Gly-Asp-Tyr($SO_3H$)-Met-Gly-Trp--Met-Asp-Phe-$NH_2$ (Compound VIII)*

(1) Synthesis of Boc-Gly-Asp-Tyr-$NHNH_2$

4.81 g (0.009 mole) of Z-Asp(OBz)-Tyr-OMe [m.p. 126-127°C. $[\alpha]_D^{24}$ = —4.8° (C = 1, methanol). Anal. Calcd. (%) for $C_{29}H_{30}N_2O_8$: C, 65.16; H, 5.66; N, 5.24. Found (%): C, 65.14; H, 5.71; N, 5.18] was dissolved in a mixture of 150 mℓ of methanol and 9 mℓ of 1N HCℓ and hydrogenated over 0.9 g of a 10% Pd-carbon catalyst at room temperature for 6 hours. Then the catalyst was filtered off, and the filtrate was distilled in vacuo to dryness. The resulting dipeptide was condensed with a mixed anhydride which had been prepared from 1.89 g of Boc-Gly--OH in the same manner as in (1) of Example 2. The product was recrystallized from ethyl acetate-ether, giving 3.09 g of Boc-Gly-Asp-Tyr-OMe; yield 71.8%, m.p. 97-99°C.

$[\alpha]_D^{24}$ = —14.1° (C = 1, methanol).

Anal. Calcd. (%) for $C_{21}H_{29}N_3O_9$: C, 53.96; H, 6.25; N, 8.99. Found (%): C, 53.95; H, 6.68; N, 8.69.

1.83 g of the above obtained tripeptide methyl ester was dissolved in 15 mℓ of methanol, then 1.08 mℓ of 90% hydrazine hydrate was added to the solution, and the mixture was stirred at room temperature for 17 hours. Ether was added to the reaction mixture in which a precipitate had formed. The precipitate was filtered, dried, and washed again with 5% acetic acid, giving 1.68 g of Boc-Gly-Asp--Tyr-$NHNH_2$; yield 92.1%, m.p. 149-151°C.

$[\alpha]_D^{25}$ = —23.6° [C = 0.5, DMF - acetic acid (1:1)].

Anal. Calcd. (%) for $C_{20}H_{29}N_5O_8$: C, 51.39; H, 6.25; N, 14.98. Found (%): C, 51.14; H, 6.37; N, 15.09.

(2) Synthesis of Boc-Gly-Asp-Tyr-Met-Gly-Trp--Met-Asp-Phe-$NH_2$

0.885 g (0.001 mole) of Boc-Met-Gly-Trp-Met--Asp-Phe-$NH_2$ [cf. (3) of Example 7] was similarly treated with 6 mℓ of trifluoroacetic acid containing 0.2 mℓ of ethandithiol and condensed with 0.701 g of the tripeptide obtained in the preceding (1), in the same manner as in (2) of Example 1 according to the azide process. The product was precipitated by adding 1N citric acid, washed with water, and recrystallized from methanol, giving 0.97 g of Boc--Gly-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-$NH_2$; yield 79.5%, m.p. 197-200°C.

$[\alpha]_D^{24}$ = —39.5° (C = 1, DMF).

Anal. Calcd. (%) for $C_{56}H_{73}N_{11}O_{16}S_2\cdot H_2O$: C, 54.31; H, 6.10; N, 12.44. Found (%): C, 54.13; H, 6.06; N, 12.18.

(3) Synthesis of Gly-Asp-Tyr($SO_3H$)-Met-Gly--Trp-Met-Asp-Phe-$NH_2$

0.488 g (0.0004 mole) of the nonapeptide obtained in the preceding (2) was dissolved in a mixture of 10 mℓ of DMF and 1 mℓ of pyridine, 0.637 g of a pyridine-sulfuric anhydride complex was added under cooling with ice, and the mixture was stirred at 0°C for 30 minutes and then at room temperature

for 17 hours. The resulting reaction mixture was concentrated in vacuo, and 20 mℓ of methanol, 20 mℓ of butanol, and 12 mℓ of 0.5 M aqueous calcium acetate solution were added to the residue. The resulting precipitate of calcium sulfate was removed by centrifugation, and the supernatant was distilled in vacuo to dryness. The residue was washed with water, and 0.503 g of Boc-Gly-Asp-Tyr($SO_4^-$)-Met--Gly-Trp-Met-Asp-Phe-$NH_2$·Ca salt was obtained.

This sulfated peptide·Ca salt was treated at 0°C for 30 minutes with 5 mℓ of trifluoroacetic acid containing 0.1 mℓ of ethandithiol. The precipitate formed by adding then anhydrous ether to the reaction mixture was filtered and purified by chromatography on a DEAE-Cellulose column (3 × 7 cm), wherein 1 ℓ of an 0.3 M ammonium carbonate buffer solution (pH 8.5) was used for elution. Thus, 0.243 g of Gly-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met--Asp-Phe-$NH_2$ (Compound VIII) was obtained as a lyophilized product; yield 50.8%.

$[\alpha]_D^{25}$ = —26.2° (C = 0.5, 1N ammonia).

Anal. Calcd. (%) for $C_{51}H_{65}N_{11}O_{17}S_3 \cdot NH_3 \cdot 5H_2O$: C, 46.85; H, 6.01; N, 12.86. Found (%): C, 46.80; H, 5.86; N, 12.81.

Amino acid analysis by acidolysis:
Asp 2.05 (2), Gly 1.99 (2), Met 2.00 (2), Tyr 0.97 (1), Phe 0.99 (1).

Infrared absorption spectrum: 1050 $cm^{-1}$ (sulfate ester).

*Example 9*

*Preparation of β-Ala-Asp-Tyr($SO_3H$)-Met-Gly--Trp-Met-Asp-Phe-$NH_2$ (Compound IX)*

(1) Synthesis of Boc-β-Ala-Asp-Tyr-$NHNH_2$

In the same manner as in (1) of Example 8, 3.25 g of Boc-β-Ala-Asp-Tyr-OMe was obtained from 4.81 g (0.009 mole) of Z-Asp(OBz)-Tyr-OMe and 2.04 g (0.011 mole) of Boc-β-Ala-OH; yield 75.0%, m.p. 88-90°C.

$[\alpha]_D^{24}$ = —22.4° (C = 1, methanol).

Anal. Calcd. (%) for $C_{22}H_{31}N_3O_8$: C, 54.88; H, 6.48; N, 8.73. Found (%): C, 54.97; H, 6.64; N, 8.42.

Also in the same manner as in (1) of Example 8, 1.56 g of the above tripeptide methyl ester was converted into a hydrazide derivative, giving 1.18 g of Boc-β-Ala-Asp-Tyr-$NHNH_2$; yield 75.7%, m.p. 183-186°C.

$[\alpha]_D^{25}$ = —38.4° [C = 0.5, DMF - acetic acid (1:1)].

Anal. Calcd. (%) for $C_{21}H_{31}N_5O_8$: C, 52.38; H, 6.49; N, 14.55. Found (%): C, 52.12; H, 6.47; N, 14.45.

(2) Synthesis of Boc-β-Ala-Asp-Tyr-Met-Gly--Trp-Met-Asp-Phe-$NH_2$

In the same manner as in (2) of Example 8, 0.977 g of Boc-β-Ala-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe--$NH_2$ was obtained according to the azide method by condensation of 0.885 g of Boc-Met-Gly-Trp--Met-Asp-Phe-$NH_2$ with 0.722 g of Boc-β-Ala-Asp--Tyr-$NHNH_2$ obtained in the preceding (1); yield 79.1%, m.p. 207-209°C.

$[\alpha]_D^{24}$ = —41.0° (C = 1, DMF).

Anal. Calcd. (%) for $C_{57}H_{75}N_{11}O_{16}S_2$: C, 55.46; H, 6.12; N, 12.48. Found (%): C, 55.65; H, 6.19; N, 12.32.

(3) Synthesis of β-Ala-Asp-Tyr($SO_3H$)-Met-Gly--Trp-Met-Asp-Phe-$NH_2$

In the same manner as in (3) of Example 8, sulfation, calcium salt formation, deprotection and purification were conducted on 0.494 g (0.4 m mole) of the nonapeptide obtained in the preceding (2), giving 0.243 g of β-Ala-Asp-Tyr($SO_3H$)-Met--Gly-Trp-Met-Asp-Phe-$NH_2$ (Compound IX); yield 50.1%.

$[\alpha]_D^{24}$ = —33.2° (C = 0.5, 1N ammonia).

Anal. Calcd. (%) for $C_{52}H_{67}N_{11}O_{17}S_3 \cdot NH_3 \cdot 4H_2O$: C, 47.92; H, 6.03; N, 12.89. Found (%): C, 48.05; H, 5.64; N, 12.66.

Amino acid analysis by acidolysis:
Asp 2.09 (2), Gly 0.98 (1), Met 2.07 (2), Tyr 1.05 (1), Phe 0.84 (1), β-Ala 0.97 (1).

Infrared absorption spectrum: 1050 $cm^{-1}$ (sulfate ester).

*Pharmacological test I*

(1) Gallbladder contracting activity

This test was in accordance with the method of G. Bertaccini et al. [Br. J. Pharmacol., 34, 291-310 (1968)].

Male Hartley guinea pigs (weighing 400-600 g) were anesthetized with urethane and fixed at the supine position. After laparotomy, the top of the gallbladder was clamped with a Serre-fine which was connected to a FD pickup (model: SB-IT, NIHON KOHDEN KOGYO Co., Ltd.), and the contraction was recorded on a recorder (model: PJB-3012, made by the above company) through a preamplifier (model: RUP-25, made by the above company). A standard solution and the invented peptide solutions were injected into the jugular vein, and the relative potency was determined from the peak height of the gallbladder contraction. Results of the test are shown in Table 1.

*Table 1* - Gallbladder contracting activity

| Peptide | | Relative potency |
|---|---|---|
| Compound | I | 0 |
| » | II | 0 |
| » | III | 8.7 |
| » | IV | 5.0 |
| » | V | < 1.0 |
| » | VI | < 1.0 |
| » | VII | < 1.0 |
| » | VIII | 97 |
| » | IX | 113 |
| CCK - 8 | | 100 |

As is shown in Table 1, Compounds VIII and IX exhibited comparable activities with CCK-8 and activities of Compounds III and IV were mild; while Compounds V, VI, and VII exhibited little activity and Compounds I and II no activity.

(2) Accelerating on pancreatic external secretion

This test was conducted in accordance with the method of Dockray [J. Physiol., 225, 679-692 (1972)]. Male Wistar rats (weighing about 280 g) were deprived for 24 hours and under anesthesia the gastric pylorus and the duodenal side end of the common hepatic duct were ligated. A canula for collection of pancreatic juice was inserted into the duct in the retrograde fashion. The bile was led into the duodenum. Varying concentrations of the control solution (CCK-8 solution) and solutions of the invented peptides in a volume of 50 μℓ were administered intravenously every one hour, thus determining the increment in the secretion rate of the pancreatic juice and protein during 30 minutes after administration, wherein the amount of the whole protein secreted was determined from the ultraviolet absorbance at 280 nm of the pancreatic juice secreted. Results thereof are shown in Figs. 1-4.

Referring to the results of the increased amount of the pancreatic juice secreted, the activities of Compounds IV, V, VI, and VIII were comparable to that of CCK-8 and the activity of Compound IX was about half of that of CCK-8, while Compounds I, II, and VII exhibited no active effect.

(3) The use of the Compounds of IV, V, VI, and VII, and the salts of the compounds for accelerating the pancreatic function will be described below

Firstly, these compounds and salts are mixed with well-known pharmaceutical carriers to prepare pharmaceutical compositions. The carriers include diluents and excipients, e.g., filler, extender, binder, wetting agent, disintegrator, and surfactant, etc. The carriers are selected depending upon a dosage form.

The Compounds of IV, V, VI, and VII, and the salts of the compounds can be contained in the pharmaceutical composition in various amount without restriction. Generally, it is preferable that these compounds and salts are contained in an amount of 1-70 wt.% based on the total composition.

The thus prepared accelerator for pancreatic function is administrated by a method depending upon a dosage form. The administration method is not restricted particularly. For example, tablets, pills, solutions, suspensions, emulsions, granules, and capsules are suitable for oral administration; parenteral solutions which contain singly the active ingredient or a mixture of conventional cosolvents such as dextrose and amino acids with the active ingredient are suitable for intravenous injection; the active ingredient is, if necessary, singly administrated intramuscularly, intracutaneously, subcutaneously, or intraperitoneally; suppositories are suitable for rectal administration; and nasal drops are suitable for nasal administration. A suitable dosage range is selected depending upon a using object, the conditions of a patient, and the like. A pharmaceutical composition containing the Compound of IV, V, VI, or VII, or a salt of the compounds is administrated to a human being of weight 60 kg for 1-4 times per day in an amount of about 4 μg to 2 mg of the compound or the salt thereof per kg of body weight per day. With regard to acute toxicity, even when the compound or a salt of the compounds was subcutaneously injected to a mouse in a dose of 2 mg/kg, no toxicity was observed.

*Example 10*
*Preparation of Glt-Ala-Tyr($SO_3H$)-Gly-Trp-Met--Asp-Phe-$NH_2$ (Compound X)*

(1) Synthesis of Glt-Ala-Tyr-Gly-Trp-Met-Asp--Phe-$NH_2$

1.00 g of Boc-Ala-Tyr-Gly-Trp-Met-Asp-Phe--$NH_2$ (m.p. 197-200°C. Anal. Calcd. (%) for $C_{48}H_{61}N_9O_{12}S$: C, 58.35; H, 6.22; N, 12.75. Found (%): C, 58.35; H, 6.43; N, 12.61) was dissolved in 5 mℓ of trifluoroacetic acid containing 0.2 mℓ of ethanedithiol and reacted at room temperature for 30 minutes. Then 100 mℓ of anhydrous ether was added to the reaction mixture, and the resulting precipitate was filtered and dried. This deprotected heptapeptide was dissolved in 20 mℓ of DMF containing 0.14 mℓ of triethylamine, and 0.40 g of glutaric anhydride was added under cooling with ice. After 17-hour stirring at 4°C, the reaction mixture was distilled in vacuo to remove the solvent, and 1N citric acid was added to the residue to form a precipitate. It was washed with water and recrystallized from 20 mℓ of methanol, giving 0.77 g of Glt-Ala-Tyr-Gly-Trp-Met-Asp-Phe-$NH_2$; yield 76.1%, m.p. 167-170°C.

$[\alpha]_D^{24} = -38.5°$ (C = 1, DMF).

Anal. Calcd. (%) for $C_{48}H_{59}N_9O_{13}S$: C, 57.53; H, 5.93; N, 12.58. Found (%): C, 57.42; H, 5.99; N, 12.22.

(2) Synthesis of Glt-Ala-Tyr($SO_3H$)-Gly-Trp--Met-Asp-Phe-$NH_2$

301 mg of the acylated peptide obtained in the preceding (1) was dissolved in a mixture of 10 mℓ of DMF and 1 mℓ of pyridine, and 477 mg of a pyridine-sulfuric anhydride complex was added under cooling with ice. The mixture was stirred at 0°C for 30 minutes and then at room temperature for 20 hours. The reaction mixture was concentrated in vacuo, 30 mℓ of 0.05 M aqueous ammonium carbonate solution was added to the residue, and the pH was adjusted to 8.5 with aqueous ammonia. The solution was purified by chromatography on a DEAE-Cellulose column (4 cm × 12 cm), wherein the adsorption and washing was carried out by using 500 mℓ of an 0.05 M buffer solution of ammonium carbonate-ammonium bicarbonate (pH 8.5) and the elution by using 700 mℓ of the same but 0.2 M buffer solution (pH 8.5). The active fractions eluted were collected, concentrated, and lyophilized, giving 163 mg of Glt-Ala-Tyr($SO_3H$)-Gly-Trp-Met-Asp--Phe-$NH_2$ (Compound X); yield 50.2%.

Anal. Calcd. (%) for $C_{48}H_{59}N_9O_{16}S_2 \cdot NH_3 \cdot 4H_2O$: C, 49.22; H, 6.02; N, 11.96. Found (%): C, 49.22; H, 5.79; N, 12.12.

Amino acid analysis by acidolysis:

Asp 1.05 (1), Gly 0.96 (1), Ala 1.02 (1), Met 0.94 (1), Tyr 1.00 (1), Phe 0.98 (1).

Infrared absorption spectrum: 1050 $cm^{-1}$ (sulfate ester).

*Example 11*

*Preparation of pGlu-Ala-Tyr($SO_3H$)-Gly-Trp-Met--Asp-Phe-$NH_2$ (Compound XI)*

(1) Synthesis of pGlu-Ala-Tyr-Gly-Trp-Met--Asp-Phe-$NH_2$

1.51 g of Boc-Gly-Trp-Met-Asp-Phe-$NH_2$ was dissolved in 5 mℓ of trifluoroacetic acid containing 0.2 mℓ of ethandithiol, and reacted at room temperature for 30 minutes. Then 100 mℓ of anhydrous ether was added to the reaction mixture, and the resulting precipitate was filtered and dried. This pentapeptide, dissolved in 20 mℓ of DMF containing 0.28 mℓ of triethylamine, was condensed with 0.76 g of pGlu-Ala-Tyr-$NHNH_2$ [m.p. 269-270°C. Anal. Calcd. (%) for $C_{17}H_{23}N_5O_6$: C, 54.10; H, 6.14; N, 18.56. Found (%): C, 54.00; H, 6.28; N, 18.74] according to the azide method wherein, the hydrazide was reacted after being converted with isoamyl nitrite into the azide. Then the solvent was distilled off in vacuo, and 1N citric acid was added to the residual liquid to form a precipitate. It was washed with water and recrystallized from dimethylformamide-methanol, giving 1.68 g of pGlu-Ala-Tyr-Gly-Trp-Met-Asp--Phe-$NH_2$; yield 84.1%, m.p. 207-209°C.

$[\alpha]_D^{24}$ = —34.9° (C = 1, DMF).

Anal. Calcd. (%) for $C_{48}H_{58}N_{10}O_{12}S$: C, 57.70; H, 5.85; N, 14.02. Found (%): C, 57.60; H, 6.15; N, 14.15.

(2) Synthesis of pGlu-Ala-Tyr($SO_3H$)-Gly-Trp--Met-Asp-Phe-$NH_2$

1.00 g of the octapeptide obtained in the preceding (1) was dissolved in a mixture of 10 mℓ of DMF and 1 mℓ of pyridine, 1.60 g of a pyridine-sulfuric anhydride complex was added under cooling with ice, and the mixture was stirred at 0°C for 30 minutes and then at room temperature for 17 hours. The resulting reaction mixture was concentrated in vacuo, 50 mℓ of an 0.05 M aqueous ammonium carbonate solution was added to the residue, and the pH was adjusted to 8.5 by adding aqueous ammonia. The solution was purified by ion exchange chromatography on a DEAE-Cellulose column (4 cm × 12 cm). The active fractions eluted were collected, concentrated, and lyophilized, giving 563 mg of pGlu-Ala-Tyr($SO_3H$)-Gly-Trp-Met-Asp-Phe-$NH_2$ (Compound XI), yield 52.2%.

Anal. Calcd. (%) for $C_{48}H_{58}N_{10}O_{15}S_2 \cdot NH_3 \cdot 4H_2O$: C, 49.35; H, 5.95; N, 13.19. Found (%): C, 49.51; H, 5.95; N, 13.02.

Amino acid analysis by acidolysis:

Asp 1.05 (1), Glu 0.96 (1), Gly 1.04 (1), Ala 0.98 (1), Met 1.00 (1), Tyr 1.03 (1), Phe 0.94 (1).

Infrared absorption spectrum: 1050 $cm^{-1}$ (sulfate ester).

*Pharmacological test II*

(1) Gastric acid secretion accelerating activity

This test was conducted with Shay rats [H. Shay et al., Gastroenterology, 5, 43 (1945)] prepared. Employing male Wistar rats (weighing 270-310 g) deprived for 24 hours, the gastric pylorus was ligated under ether-anesthesia. Immediately thereafter, the invented peptides and tetragastrin as a control were administered intravenously. Gastric juice was withdrawn 2 hours after administration, and its amount and the total amount of acid secreted were measured to determine the relative potency based on tetragastrin. Results of the test are shown in Table 2.

*Table 2* - Gastric acid secretion accelerating activity

| Peptide | Relative potency |
|---|---|
| Compound X | 6.1 |
| » XI | 2.0 |
| Tetragastrin | 1.0 |

(2) Pancreatic external secretion accelerating activity

The test was conducted in the same manner as stated already. The increment in the secretion rate of the protein in the pancreatic during 30 minutes after administration of the test solution was determined from the ultraviolet absorbance at 280 nm of the pancreatic juice. The found values were compared with that of tetragastrin. Results thereof are shown in Table 3.

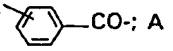
*Table 3* - Pancreatic external secretion accelerating activity

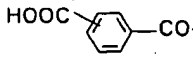
| Peptide | Relative potency |
|---|---|
| Compound X | 258.8 |
| » XI | 50.0 |
| Tetragastrin | 1.0 |

**Claims**

1. Novel peptides represented by the following formula

$R_1$-A-B-C-D-Trp-Met-Y (1)

wherein $R_1$ denotes pGlu, X-$R_2$-CO-, X being carboxyl or amino and $R_2$ being lower alkylene of 1-6 carbon atoms, or  ; A denotes Asp, Ala, or merely a chemical bond; B denotes Tyr($SO_3H$) or Phe($NHSO_3H$); C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes $NH_2$, Asp-$NH_2$, or Asp-Phe-$NH_2$; with

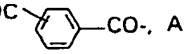
the proviso that Y is $NH_2$ or Asp-$NH_2$ when $R_1$ is pGlu, HOOC-$R_2$-CO-, or  , B is Tyr($SO_3H$), C is Met, and D is Gly, and pharmacologically acceptable salts thereof.

2. Novel peptides and salts according to claim 1, wherein $R_1$ is $NH_2$-$R_2$-CO-, $R_2$ being lower alkylene of 1-6 carbon atoms.

3. Novel peptides and salts according to claim 1 or 2, wherein B is Phe($NHSO_3H$).

4. Novel peptides and salts according to any preceding claim, wherein D is D-Ala or D-Trp.

5. Novel peptides and salts according to any preceding claim, wherein Y is $NH_2$ or Asp-$NH_2$.

6. The novel peptides and pharmacologically acceptable salts thereof according to any preceding claim, wherein $R_1$ is pGlu, X-$R_2$-CO-, X being carboxyl group or amino group and $R_2$ being lower alkylene group of 1-6 carbon atoms, or  , A is Ala, and C is merely a chemical bond.

7. A novel peptide according to claim 1, selected from:

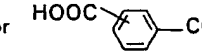
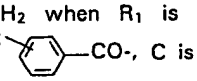
Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$,
Suc-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$,
Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-$NH_2$,
Suc-Tyr($SO_3H$)-Met-D-Ala-Trp-Met-Asp-Phe-$NH_2$,
Suc-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Suc-Phe($NHSO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
Gly-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
β-Ala-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
pGlu-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Glt-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Pht-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Glt-Tyr($SO_3H$)-Met-D-Ala-Trp-Met-Asp-Phe-$NH_2$,
Glt-Phe($NHSO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
Glt-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$,
Glt-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-$NH_2$,
Gly-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
β-Ala-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
Glt-Ala-Tyr($SO_3H$)-Gly-Trp-Met-Asp-Phe-$NH_2$, and
pGlu-Ala-Tyr($SO_3H$)-Gly-Trp-Met-Asp-Phe-$NH_2$.

8. A novel peptide according to claim 1 selected from:
Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$,
Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-$NH_2$,
Suc-Tyr($SO_3H$)-Met-D-Ala-Trp-Met-Asp-Phe-$NH_2$,
Suc-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Suc-Phe($NHSO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
Glt-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Pht-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Gly-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
β-Ala-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
Glt-Ala-Tyr($SO_3H$)-Gly-Trp-Met-Asp-Phe-$NH_2$, and
pGlu-Ala-Tyr($SO_3H$)-Gly-Trp-Met-Asp-Phe-$NH_2$.

9. A pharmacologically acceptable peptide salt according to any preceding claim selected from alkali metal salts, alkali earth metal salts, organic amine salts, and ammonium salts.

10. A process for the preparation of novel peptides represented by the following formula

$R_1$-A-Tyr($SO_3H$)-C-D-Trp-Met-Y (6)

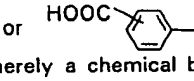
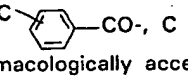
wherein; $R_1$ denotes pGlu, X-$R_2$-CO-, X being carboxyl or amino and $R_2$ being lower alkylene of 1-6 carbon atoms, or  ; A denotes Asp, Ala, or merely a chemical bond; C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes $NH_2$, Asp-$NH_2$, or Asp-Phe-$NH_2$; with the proviso that Y is $NH_2$ or Asp-$NH_2$ when $R_1$ is pGlu, HOOC-$R_2$-CO-, or  , C is Met, and D is Gly, and pharmacologically acceptable salts thereof, characterized in that the Tyr-residue in the peptide of the following formula

$R_1$-A-Tyr-C-D-Trp-Met-Y (2)

wherein; $R_1$, A, C, D, and Y are the same as defined above, or the peptide in which active groups such as amino groups are protected by suitable protecting groups, is subjected to the sulfate-ester reaction to convert the Tyr-residue to Tyr($SO_3H$) residue.

11. A process for the preparation of novel peptides represented by the following formula

$R_1$-A-Tyr($SO_3H$)-C-D-Trp-Met-Y (6)

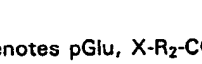
wherein; $R_1$ denotes pGlu, X-$R_2$-CO-, X being carboxyl or amino and $R_2$ being lower alkylene of 1-6 carbon atoms, or  ; A denotes Asp, Ala, or merely a chemical bond; C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes $NH_2$, Asp-$NH_2$, or Asp-Phe-$NH_2$; with the proviso that Y is $NH_2$ or Asp-$NH_2$ when $R_1$ is pGlu, HOOC-$R_2$-CO-, or  , C is Met, and D is Gly, and pharmacologically acceptable salts thereof, characterized in that a sulfo group of a protected peptide amide sulfate ester obtained by the sulfate-ester reaction of the Tyr residue in the protected peptide of the following formula

$R_1$-A-Tyr-C-D-Trp-Met-Y (2)

wherein; $R_1$, A, C, D, and Y are the same as defined above, is converted to a salt of divalent metal such as Ca, Zn, and the like to stabilize said protected peptide amide sulfate ester, and thereafter said protected peptide amide sulfate ester is deprotected.

12. A process for the preparation of novel peptides represented by the following formula

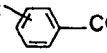
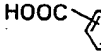
$R_1$-A-Phe($NHSO_3H$)-C-D-Trp-Met-Y (6)

wherein; $R_1$ denotes pGlu, X-$R_2$-CO-, X being carboxyl or amino and $R_2$ being lower alkylene of 1-6 carbon atoms, or  ; A denotes Asp, Ala, or merely a chemical bond; C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp, and pharmacologically acceptable salts

thereof, characterized in that a divalent metal salt of a sulfonated p-amino phenylalanine derivative is prepared, and a peptide chain is elongated by use of said divalent metal salt.

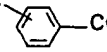
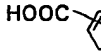
13. An accelerator for pancreatic function which contains a novel peptide represented by the following formula

$R_1$-A-B-C-D-Trp-Met-Y (1)

wherein; $R_1$ denotes pGlu, X-$R_2$-CO-, X being carboxyl or amino and $R_2$ being lower alkylene of 1-6 carbon atoms, or  ; A denotes Asp, Ala, or merely a chemical bond; B denotes Tyr($SO_3H$) or Phe($NHSO_3H$); C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes $NH_2$, Asp-$NH_2$, or Asp-Phe-$NH_2$; with the proviso that Y is $NH_2$ or Asp-$NH_2$ when $R_1$ is pGlu, HOOC-$R_2$-CO-, or  , B is Tyr($SO_3H$), C is Met, and D is Gly, and pharmacologically acceptable salts thereof, as an effective component.

14. A reagent for pancreatic function tests or a contrast medium for gallbladder which contains a novel peptide represented by the following formula

$R_1$-A-B-C-D-Trp-Met-Y (1)

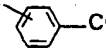
wherein; $R_1$ denotes pGlu, X-$R_2$-CO-, X being carboxyl or amino and $R_2$ being lower alkylene of 1-6 carbon atoms, or  ; A denotes Asp, Ala, or merely a chemical bond; B denotes Tyr($SO_3H$) or Phe($NHSO_3H$); C denotes Met or merely a chemical bond; D denotes Gly, D-Ala, or D-Trp; and Y denotes $NH_2$, Asp-$NH_2$, or Asp-Phe-$NH_2$; with the proviso that Y is $NH_2$ or Asp-$NH_2$ when $R_1$ is pGlu, HOOC-$R_2$-CO-, or  , B is Tyr($SO_3H$), C is Met, and D is Gly, and pharmacologically acceptable salts thereof as an effective component.

**Patentansprüche**

1. Neue Peptide der folgenden Formel

$R_1$-A-B-C-D-Trp-Met-Y (1)

worin bedeuten:

$R_1$ pGlu, X-$R_2$-CO-, worin X Carboxyl oder Amino und $R_2$ niederes Alkylen mit 1-6 Kohlenstoffatomen bedeuten, oder  ;

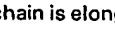
A Asp, Ala oder lediglich eine chemische Bindung;

B Tyr($SO_3H$) oder Phe($NHSO_3H$);

C Met oder lediglich eine chemische Bindung;

D Gly, D-Ala oder D-Trp; und

Y $NH_2$, Asp-$NH_2$ oder Asp-Phe-$NH_2$;

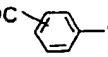
mit der Massgabe, dass Y die Bedeutung von $NH_2$ oder Asp-$NH_2$ hat, wenn $R_1$ pGlu, HOOC-$R_2$-CO- oder  , B Tyr($SO_3H$), C Met und D Gly bedeuten, und pharmakologisch annehmbare Salze hiervon.

2. Neue Peptide und Salze nach Anspruch 1, wobei $R_1$ die Bedeutung von $NH_2$-$R_2$-CO- hat, worin $R_2$ niederes Alkylen mit 1-6 Kohlenstoffatomen ist.

3. Neue Peptide und Salze nach Anspruch 1 oder 2, wobei B die Bedeutung von Phe($NHSO_3H$) hat.

4. Neue Peptide und Salze nach einem der vorhergehenden Ansprüche, wobei D die Bedeutung D-Ala oder D-Trp hat.

5. Neue Peptide und Salze nach einem der vorhergehenden Ansprüche, wobei Y die Bedeutung $NH_2$ oder Asp-$NH_2$ hat.

6. Neue Peptide und pharmakologisch annehmbare Salze hiervon nach einem der vorhergehenden Ansprüche, wobei $R_1$ die Bedeutung p-Glu, X-$R_2$--CO-, worin X eine Carboxyl- oder Aminogruppe und $R_2$ eine niedere Alkylengruppe mit 1-6 Kohlenstoffatomen bedeuten, oder  , hat, A Ala und C lediglich eine chemische Bindung bedeuten.

7. Neues Peptid nach Anspruch 1, gewählt aus:

Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$,
Suc-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$,
Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-$NH_2$,
Suc-Tyr($SO_3H$)-Met-D-Ala-Trp-Met-Asp-Phe-$NH_2$,
Suc-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Suc-Phe($NHSO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
Gly-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
β-Ala-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
pGlu-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Glt-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Pht-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Glt-Tyr($SO_3H$)-Met-D-Ala-Trp-Met-Asp-Phe-$NH_2$,
Glt-Phe($NHSO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
Glt-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$,
Glt-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-$NH_2$,
Gly-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
β-Ala-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
Glt-Ala-Tyr($SO_3H$)-Gly-Trp-Met-Asp-Phe-$NH_2$, und
pGlu-Ala-Tyr($SO_3H$)-Gly-Trp-Met-Asp-Phe-$NH_2$.

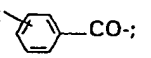
8. Neues Peptid nach Anspruch 1, gewählt aus:
Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$,
Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-$NH_2$,
Suc-Tyr($SO_3H$)-Met-D-Ala-Trp-Met-Asp-Phe-$NH_2$,
Suc-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Suc-Phe($NHSO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
Glt-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Pht-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Gly-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
β-Ala-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
Glt-Ala-Tyr($SO_3H$)-Gly-Trp-Met-Asp-Phe-$NH_2$, und
pGlu-Ala-Tyr($SO_3H$)-Gly-Trp-Met-Asp-Phe-$NH_2$.

9. Pharmakologisch annehmbares Peptidsalz nach einem der vorangehenden Ansprüche, gewählt aus Alkalimetallsalzen, Erdalkalimetallsalzen, organischen Aminsalzen und Ammoniumsalzen.

10. Verfahren zur Herstellung neuer Peptide der folgenden Formel

$R_1$-A-Tyr($SO_3H$)-C-D-Trp-Met-Y (6)

worin bedeuten:

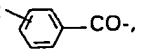
$R_1$ pGlu, X-$R_2$-CO-, worin X Carboxyl oder Amino und $R_2$ niederes Alkylen mit 1-6 Kohlenstoffatomen bedeuten, oder  ;

A Asp, Ala oder lediglich eine chemische Bindung;

C Met oder lediglich eine chemische Bindung;

D Gly, D-Ala oder D-Trp; und

Y $NH_2$, Asp-$NH_2$ oder Asp-Phe-$NH_2$;

mit der Massgabe, dass Y die Bedeutung $NH_2$ oder Asp-$NH_2$ hat, wenn $R_1$ pGlu, HOOC-$R_2$-CO- oder  , C Met und D Gly bedeuten,

und pharmakologisch annehmbarer Salze hiervon, dadurch gekennzeichnet, dass der Tyr-Rest in dem Peptid der folgenden Formel

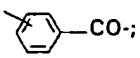
$R_1$-A-Tyr-C-D-Trp-Met-Y (2)

worin $R_1$, A, C, D und Y in gleicher Weise wie oben definiert sind, oder dem Peptid, in dem aktive Gruppen, wie etwa Aminogruppen, durch geeignete Schutzgruppen geschützt sind, der Sulfat-Ester-Reaktion unterzogen wird, um den Tyr-Rest in den Tyr($SO_3H$)-Rest umzuwandeln.

11. Verfahren zur Herstellung neuer Peptide der folgenden Formel

$R_1$-A-Tyr($SO_3H$)-C-D-Trp-Met-Y (6)

worin bedeuten:

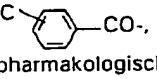
$R_1$ pGlu, X-$R_2$-CO-, worin X Carboxyl oder Amino und $R_2$ niederes Alkylen mit 1-6 Kohlenstoffatomen bedeuten, oder  ;

A Asp, Ala oder lediglich eine chemische Bindung;

C Met oder lediglich eine chemische Bindung;

D Gly, D-Ala oder D-Trp; und

Y $NH_2$, Asp-$NH_2$ oder Asp-Phe-$NH_2$;

mit der Massgabe, dass Y die Bedeutung $NH_2$ oder Asp-$NH_2$ hat, wenn $R_1$ pGlu, HOOC-$R_2$-CO- oder  , C Met und D Gly bedeuten,

und pharmakologisch annehmbarer Salz hiervon, dadurch gekennzeichnet, dass eine Sulfogruppe eines geschützten Peptidamid-Sulfatesters, erhalten durch die Sulfat-Ester-Reaktion des Tyr-Restes in dem geschützten Peptid der folgenden Formel

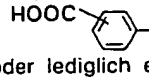
$R_1$-A-Tyr-C-D-Trp-Met-Y (2)

worin $R_1$, A, C, D und Y in gleicher Weise wie oben definiert sind, in ein Salz eines zweiwertigen Metalls, wie etwa Ca, Zn und dergleichen überführt wird, um den geschützten Peptidamid-Sulfat-Ester zu stabilisieren und danach der geschützte Peptidamid-Sulfat-Ester von der Schutzgruppe befreit wird.

12. Verfahren zur Herstellung neuer Peptide der folgenden Formel

$R_1$-A-Phe($NHSO_3H$)-C-D-Trp-Met-Y (6)

worin bedeuten:

$R_1$ pGlu, X-$R_2$-CO-, worin X Carboxyl oder Amino und $R_2$ niederes Alkylen mit 1-6 Kohlenstoffatomen bedeuten, oder  ;

A Asp, Ala oder lediglich eine chemische Bindung;

C Met oder lediglich eine chemische Bindung;

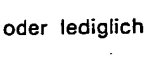
D Gly, D-Ala oder D-Trp,

und pharmakologisch annehmbarer Salze hiervon, dadurch gekennzeichnet, dass ein zweiwertiges Metallsalz eines sulfonierten p-Aminophenylalanin-Derivates hergestellt wird und eine Peptidkette durch Verwendung des zweiwertigen Metallsalzes verlängert wird.

13. Beschleunigung für die Pankreasfunktion, enthaltend ein neues Peptid der folgenden Formel

$R_1$-A-B-C-D-Trp-Met-Y (1)

worin bedeuten:

$R_1$ pGlu, X-$R_2$-CO-, worin X Carboxyl oder Amino und $R_2$ niederes Alkylen mit 1 bis 6 Kohlenstoffatomen bedeuten, oder  ;

A Asp, Ala oder lediglich eine chemische Bindung;

B Tyr($SO_3H$) oder Phe($NHSO_3H$);

C Met oder lediglich eine chemische Bindung;

D Gly, D-Ala oder D-Trp; und

Y $NH_2$, Asp-$NH_2$ oder Asp-Phe-$NH_2$;

mit der Massgabe, Dass Y die Bedeutung $NH_2$ oder Asp-$NH_2$ hat, wenn $R_1$ pGlu, HOOC-$R_2$-CO- oder  ,

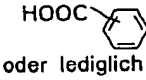
B Tyr($SO_3H$), C Met und D Gly bedeuten,

und pharmakologisch annehmbare Salze hiervon als Wirkstoffkomponente.

14. Reagenz für Pankreasfunktionsprüfungen oder Kontrastmedium für die Gallenblase, enthaltend ein neues Peptid der folgenden Formel

$R_1$-A-B-C-D-Trp-Met-Y (1)

worin bedeuten:

$R_1$ pGlu, X-$R_2$-CO-, worin X Carboxyl oder Amino und $R_2$ niederes Alkylen mit 1-6 Kohlenstoffatomen bedeuten, oder  ;

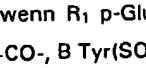
A Asp, Ala oder lediglich eine chemische Bindung;

B Tyr($SO_3H$) oder Phe($NHSO_3H$);

C Met oder lediglich eine chemische Bindung;

D Gly, D-Ala oder D-Trp; und

Y $NH_2$. Asp-$NH_2$ oder Asp-Phe-$NH_2$;

mit der Massgabe, dass Y die Bedeutung $NH_2$ oder Asp-$NH_2$ hat, wenn $R_1$ p-Glu, HOOC-$R_2$-CO- oder  , B Tyr($SO_3H$), C Met und D Gly, bedeuten,

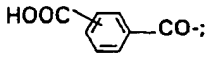
und pharmakologisch annehmbare Salze hiervon als Wirkstoffkomponente.

## Revendications

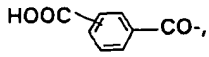
1. Peptides nouveaux représentés par la formule suivante

$R_1$-A-B-C-D-Trp-Met-Y (1)

dans laquelle $R_1$ représente pGlu, X-$R_2$-CO-, X étant un groupe carboxyle ou amino et $R_2$ étant un groupe alkylène inférieur ayant 1 à 6 atomes de carbone, ou un groupe



A représente Asp, Ala ou simplement une liaison chimique; B représente Tyr($SO_3H$) ou Phe($NHSO_3H$); C représente Met ou simplement une liaison chimique; D représente Gly, D-Ala ou D-Trp; et Y représente $NH_2$, Asp-$NH_2$ ou Asp-Phe-$NH_2$; sous réserve que Y représente $NH_2$ ou Asp-$NH_2$ lorsque $R_1$ est un groupe pGlu, HOOC-$R_2$-CO- ou



B représente Tyr($SO_3H$), C représente Met et D représente Gly, et leurs sels pharmacologiquement acceptables.

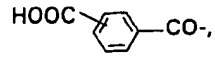
2. Peptides nouveaux et leurs sels suivant la revendication 1, dans lesquels $R_1$ représente $NH_2$--$R_2$-CO-, $R_2$ étant un groupe alkylène inférieur ayant 1 à 6 atomes de carbone.

3. Peptides nouveaux et leurs sels suivant la revendication 1 ou 2, dans lesquels B représente Phe($NHSO_3H$).

4. Peptides nouveaux et leurs sels suivant l'une quelconque des revendications précédentes, dans lesquels D représente D-Ala ou D-Trp.

5. Peptides nouveaux et leurs sels suivant l'une quelconque des revendications précédentes, dans lesquels Y représente $NH_2$ ou Asp-$NH_2$.

6. Peptides nouveaux et leurs sels pharmacologiquement acceptables suivant l'une quelconque des revendications précédentes, dans lesquels $R_1$ représente p-Glu, X-$R_2$-CO-, X étant un groupe carboxyle ou amino et $R_2$ étant un groupe alkylène inférieur ayant 1 à 6 atomes de carbone, ou un groupe



A représente Ala et C est simplement une liaison chimique.

7. Peptide nouveau suivant la revendication 1, choisi entre:
Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$,
Suc-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$,
Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-$NH_2$,
Suc-Tyr($SO_3H$)-Met-D-Ala-Trp-Met-Asp-Phe-$NH_2$,
Suc-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Suc-Phe($NHSO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
Gly-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
β-Ala-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
pGlu-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Glt-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Pht-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Glt-Tyr($SO_3H$)-Met-D-Ala-Trp-Met-Asp-Phe-$NH_2$,
Glt-Phe($NHSO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
Glt-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$,
Glt-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-$NH_2$,
Gly-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
β-Ala-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
Glt-Ala-Tyr($SO_3H$)-Gly-Trp-Met-Asp-Phe-$NH_2$, et
pGlu-Ala-Tyr($SO_3H$)-Gly-Trp-Met-Asp-Phe-$NH_2$.

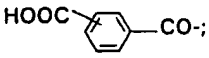
8. Peptide nouveau suivant la revendication 1, choisi entre:
Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-$NH_2$,
Suc-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-$NH_2$,
Suc-Tyr($SO_3H$)-Met-D-Ala-Trp-Met-Asp-Phe-$NH_2$,
Suc-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Suc-Phe($NHSO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
Glt-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Pht-Tyr($SO_3H$)-Met-D-Trp-Trp-Met-Asp-Phe-$NH_2$,
Gly-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
β-Ala-Asp-Tyr($SO_3H$)-Met-Gly-Trp-Met-Asp-Phe-$NH_2$,
Glt-Ala-Tyr($SO_3H$)-Gly-Trp-Met-Asp-Phe-$NH_2$, et
pGlu-Ala-Tyr($SO_3H$)-Gly-Trp-Met-Asp-Phe-$NH_2$.

9. Sel de peptide pharmacologiquement acceptable suivant l'une quelconque des revendications précédentes, choisi entre des sels de métaux alcalins, des sels de métaux alcalino-terreux, des sels d'amines organiques et des sels d'ammonium.

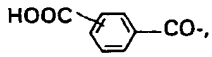
10. Procédé de préparation de peptides nouveaux représentés par la formule suivante:

$R_1$-A-Tyr($SO_3H$)-C-D-Trp-Met-Y (6)

dans laquelle $R_1$ représente pGlu, X-$R_2$-CO-, X étant un groupe carboxyle ou amino et $R_2$ étant un groupe alkylène inférieur ayant 1 à 6 atomes de carbone, ou un groupe



A représente Asp, Ala ou simplement une liaison chimique; C représente Met ou simplement une liaison chimique; D représente Gly, D-Ala ou D-Trp; et Y représente $NH_2$, Asp-$NH_2$ ou Asp-Phe-$NH_2$; sous réserve que Y représente $NH_2$ ou Asp-$NH_2$ lorsque $R_1$ représente pGlu, HOOC-$R_2$-CO- ou



C représente Met et D représente Gly, et de leurs sels pharmacologiquement acceptables, caractérisé en ce que le résidu Tyr du peptide de formule suivante

$R_1$-A-Tyr-C-D-Trp-Met-Y (2)

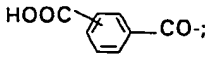
dans laquelle $R_1$, A, C, D et Y sont tels que définis ci-dessus, ou le peptide dont les groupes actifs tels que des groupes amino sont protégés par des groupes protecteurs convenables, est soumis à la réaction d'estérification sulfurique pour convertir le résidu Tyr en résidu Tyr($SO_3H$).

11. Procédé de préparation des peptides nouveaux représentés par la formule suivante

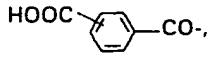
$R_1$-A-Tyr($SO_3H$)-C-D-Trp-Met-Y (6)

dans laquelle $R_1$ représente pGlu, X-$R_2$-CO-, X étant un groupe carboxyle ou amino et $R_2$ étant un groupe

alkylène inférieur de 1 à 6 atomes de carbone, ou un groupe



A désigne un groupe Asp, Ala ou simplement une liaison chimique; C désigne Met ou simplement une liaison chimique; D représente Gly, D-Ala ou D-Trp; et Y représente $NH_2$, Asp-$NH_2$ ou Asp-Phe-$NH_2$; sous réserve que Y soit un groupe $NH_2$ ou Asp-$NH_2$ lorsque $R_1$ représente pGlu, HOOC-$R_2$-CO- ou



C représente Met et D représente Gly, et de leurs sels pharmacologiquement acceptables, caractérisé en ce qu'un groupe sulfo d'un ester sulfurique d'amide peptidique protégé obtenu par la réaction d'estérification sulfurique du résidu Tyr dans le peptide protégé de formule suivante

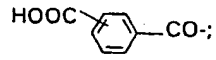
$R_1$-A-Tyr-C-D-Trp-Met-Y (2)

où $R_1$, A, C, D et Y sont tels que définis ci-dessus, est converti en un sel d'un métal divalent tel que Ca, Zn, etc. pour stabiliser ledit ester sulfurique d'amide peptidique protégé, puis cet ester est débarassé de sa protection.

12. Procédé de préparation de peptides nouveaux représentés par la formule suivante

$R_1$-A-Phe($NHSO_3H$)-C-D-Trp-Met-Y (6)

dans laquelle $R_1$ représente pGlu, X-$R_2$-CO-, X étant un groupe carboxyle ou amino et $R_2$ étant un groupe alkylène inférieur ayant 1 à 6 atomes de carbone, ou un groupe

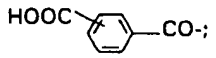


A représente Asp, Ala ou simplement une liaison chimique; C désigne Met ou simplement une liaison chimique; D désigne Gly, D-Ala ou D-Trp, et de leurs sels pharmacologiquement acceptables, caractérisé en ce qu'on prépare un sel de métal divalent d'un dérivé sulfoné de p-aminophénylalanine et on allonge une chaîne peptidique en utilisant ledit sel métallique divalent.

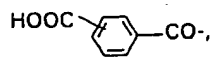
13. Accélérateur pour la fonction pancréatique, qui contient un peptide nouveau représenté par la formule suivante

$R_1$-A-B-C-D-Trp-Met-Y (1)

dans laquelle $R_1$ représente pGlu, X-$R_2$-CO-, X étant un groupe carboxyle ou amino et $R_2$ étant un groupe alkylène inférieur ayant 1 à 6 atomes de carbone, ou un groupe



A représente Asp, Ala ou simplement une liaison chimique; B désigne Tyr($SO_3H$) ou Phe($NHSO_3H$); C représente Met ou simplement une liaison chimique; D représente Gly, D-Ala ou D-Trp; et Y représente $NH_2$, Asp-$NH_2$ ou Asp-Phe-$NH_2$; sous réserve que Y soit un groupe $NH_2$ ou Asp-$NH_2$ lorsque $R_1$ représente pGlu, HOOC-$R_2$-CO-, ou

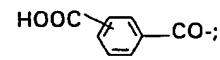


B représente Tyr($SO_3H$), C représente Met et D représente Gly, et ses sels pharmacologiquement acceptables, comme composant actif.

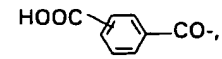
14. Réactif destiné à des tests sur la fonction pancréatique ou milieu de contraste pour la vésicule biliaire, qui contient un peptide nouveau représenté par la formule suivante

$R_1$-A-B-C-D-Trp-Met-Y (1)

dans laquelle $R_1$ représente pGlu, X-$R_2$-CO-, X étant un groupe carboxyle ou amino et $R_2$ étant un groupe alkylène inférieur ayant 1 à 6 atomes de carbone, ou un groupe



A représente Asp, Ala ou simplement une liaison chimique; B représente Tyr($SO_3H$) ou Phe($NHSO_3H$); C représente Met ou simplement une liaison chimique; D représente Gly, D-Ala ou D-Trp; et Y représente $NH_2$, Asp-$NH_2$ ou Asp-Phe-$NH_2$; sous réserve que Y soit un groupe $NH_2$ ou Asp-$NH_2$ lorsque $R_1$ représente pGlu, HOOC-$R_2$-CO- ou



B représente Tyr($SO_3H$), C représente Met et D représente Gly, et ses sels pharmacologiquement acceptables, comme composant actif.

FIG. 1

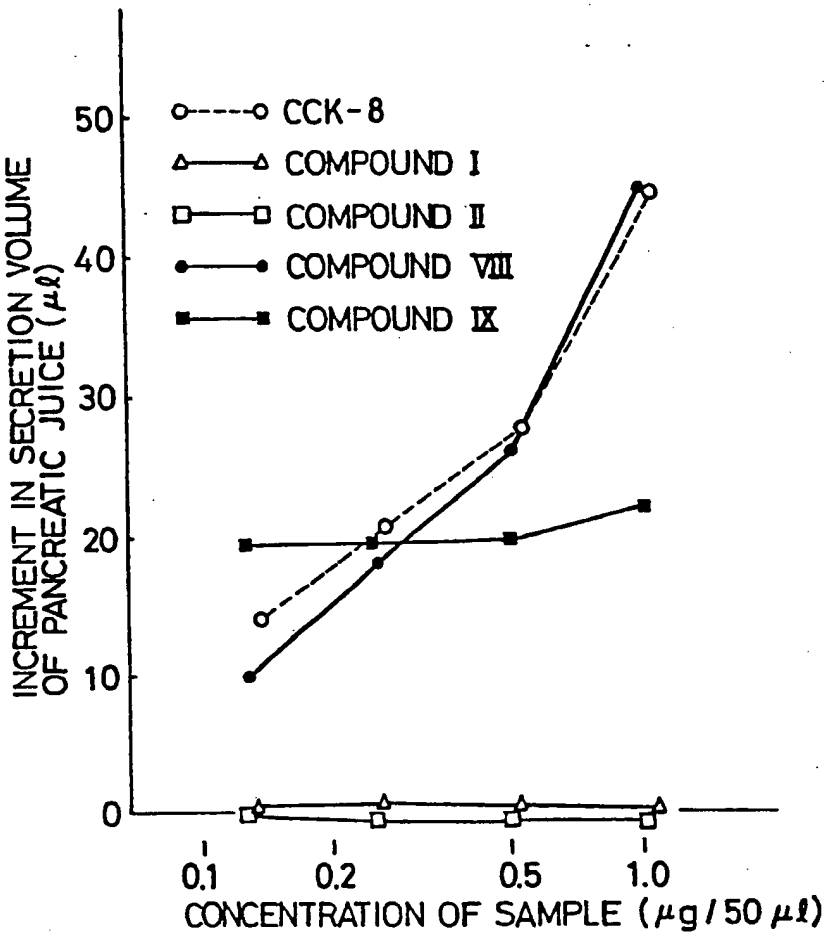

CCK-8
COMPOUND I
COMPOUND II
COMPOUND VIII
COMPOUND IX
INCREMENT IN SECRETION VOLUME OF PANCREATIC JUICE (μl)
50
40
30
20
10
0
0.1
0.2
0.5
1.0
CONCENTRATION OF SAMPLE (μg/50 μl)

FIG. 2

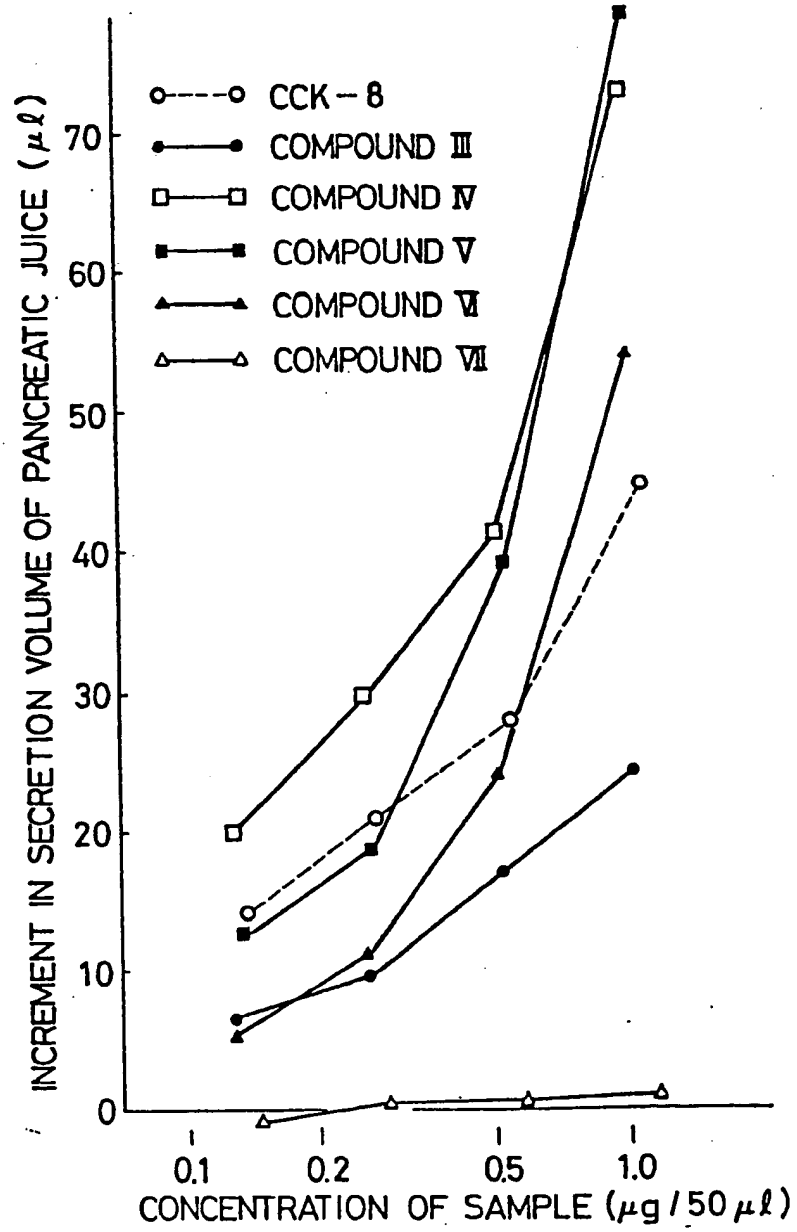

CCK-8
COMPOUND III
COMPOUND IV
COMPOUND V
COMPOUND VI
COMPOUND VII
INCREMENT IN SECRETION VOLUME OF PANCREATIC JUICE (μl)
0
10
20
30
40
50
60
70
0.1
0.2
0.5
1.0
CONCENTRATION OF SAMPLE (μg/50μl)

FIG. 3

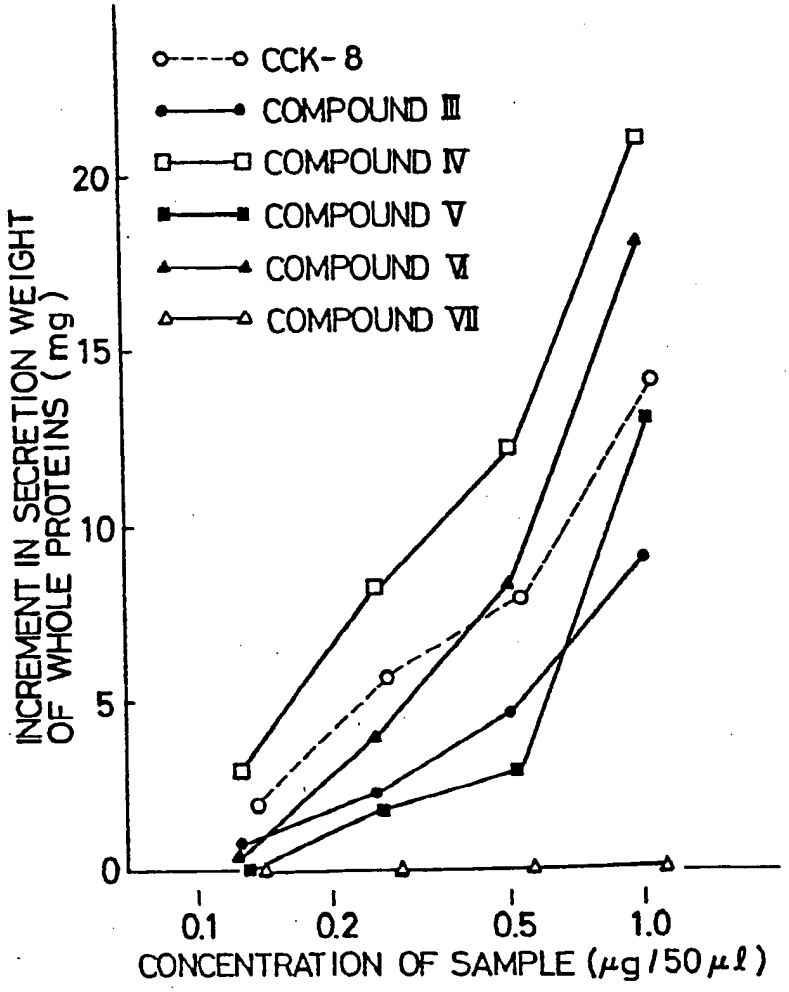
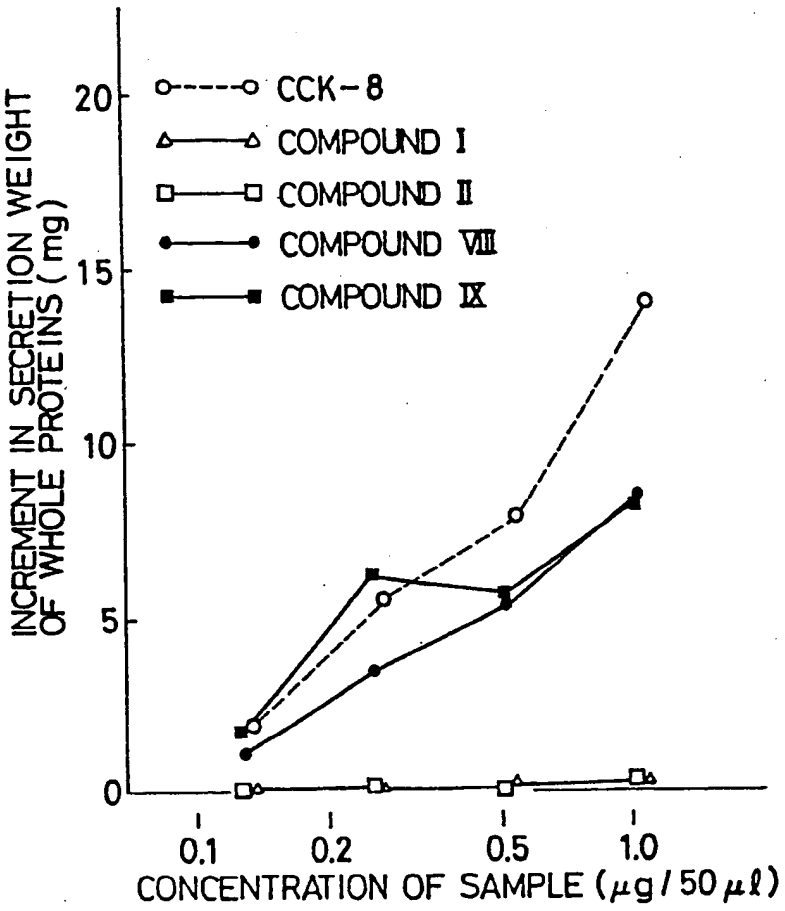

CCK-8
COMPOUND III
COMPOUND IV
COMPOUND V
COMPOUND VI
COMPOUND VII
INCREMENT IN SECRETION WEIGHT OF WHOLE PROTEINS (mg)
0
5
10
15
20
0.1
0.2
0.5
1.0
CONCENTRATION OF SAMPLE (μg/50μl)

FIG. 4


INCREMENT IN SECRETION WEIGHT OF WHOLE PROTEINS (mg)
20
15
10
5
0
CCK-8
COMPOUND I
COMPOUND II
COMPOUND VIII
COMPOUND IX
0.1
0.2
0.5
1.0
CONCENTRATION OF SAMPLE (μg/50μl)